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# THE AMERICAN JOURNAL OF PHYSIOLOGY

VOLUME 157

April-June 1949

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THE AMERICAN PHYSIOLOGICAL SOCIETY  
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BALTIMORE, MARYLAND**  
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# THE AMERICAN JOURNAL OF PHYSIOLOGY

*Published by*  
THE AMERICAN PHYSIOLOGICAL SOCIETY

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VOLUME 157

April 1949

NUMBER I

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## EFFECT OF INCREASED RENAL VENOUS PRESSURE ON RENAL FUNCTION<sup>1</sup>

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AND HENRY P. WARD<sup>3</sup>

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THE formation of edema in cardiac failure was assumed for many years to be secondary to an increase in venous pressure. According to this hypothesis the major factor causing water and salt retention was extrarenal; the increased hydrostatic pressure forced fluid out of the vascular compartment into the tissue spaces and thus made this fluid unavailable for excretion. The lesser, or renal factor, leading to the oliguria of cardiac failure was supposedly the result of renal congestion caused by the increased venous pressure (1). In 1913 Rowntree, Fitz and Geraghty (2) studied the effects of chronic passive congestion of the kidney on renal function in dogs. They applied a band about the left renal vein and collected urine from the two kidneys separately through ureteral catheters. They observed in some dogs little change in urine flow on the left but a 20 to 40 per cent reduction in phenol-sulphonphthalein (PSP) excretion and a diminished urine chloride concentration and chloride output. When they performed a right nephrectomy and then banded the left renal vein, there was a reduction in salt output without change in PSP excretion and no consistent change in urine flow.

In 1937 Winton (3) reviewed the physical factors governing urine flow. On the basis of his experiments in animals, as well as the work of others, he concluded that

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Received for publication January 6, 1949.

<sup>1</sup> Aided by grants from the Life Insurance Medical Research Fund and the U. S. Public Health Service.

<sup>2</sup> Post doctorate research fellow of the U. S. Public Health Service.

<sup>3</sup> Student fellow of the Life Insurance Medical Research Fund.

compression of the renal vein with subsequent renal congestion leads to a reduction in urine flow and chloride excretion. The findings were believed to result from a partial obstruction to the flow of urine through the tubules by the increased intra-renal pressure. Studies have been conducted on the effects of increased intra-abdominal pressure on renal function (4-7). Reduction in urine flow and sodium excretion (8) have been demonstrated but the results are difficult to evaluate since the pressure in the renal pelvis as well as the pressure in the renal vein were increased.

Since the introduction of renal-clearance techniques the mechanisms involved in the renal retention of sodium in cardiac failure have received considerable attention. Warren and Stead (9), Merrill (10), Mokotoff, Ross and Leiter (11) and Stead, Warren and Brannon (12), have suggested that in many patients reduced sodium excretion is related to a reduction in glomerular filtration rate, especially following muscular activity (13). Other workers believe that the decreased sodium excretion is not entirely related to filtration rate but is related to an increased rate of sodium reabsorption (14-17). In accordance with classical concepts elevated venous pressure has been suggested as a possible cause (14, 18). The present studies were undertaken to determine by the newer techniques available what effect the single variable of increased renal venous pressure has on renal function.

#### METHODS

Twenty-two male or female dogs weighing 10 to 32 kg. were used. They were anesthetized with sodium pentobarbital administered intravenously in doses of 30 to 35 mg/kg. Renal function was measured separately and simultaneously in the two kidneys by means of clearance techniques. During the experiment the pressure was raised only in the left renal vein. The right kidney thus served as a control.

Certain operative procedures were necessary to prepare the animal for study. The abdomen was opened by midline incision. Both ureters were cannulated with plastic tubing in order to collect urine from the two kidneys separately. The left renal venous pressure was measured with a saline manometer through a no. 9 or 10 venous catheter which had been inserted through a small incision into the right jugular vein and thence manipulated into the left renal vein. A constant infusion of isotonic saline solution flowed slowly through the catheter to prevent blockage by blood clot. The pressure in the left renal vein was raised by means of a specially constructed screw-clamp. Extension arms on the clamp made it possible to compress the renal vein and release the compression without disturbing the abdominal contents.

When the operative procedure was completed, urine was collected separately from the two kidneys during periods of equal duration ranging from 5 to 20 minutes. After two or three initial control periods the pressure in the left renal vein was raised by 50 to 420 mm. saline above the initial level which ranged around 100 mm. saline. The elevation in venous pressure was maintained for two to four periods. The clamp was then released and renal function studied for two or three additional periods at the control venous pressure. The mean arterial blood pressure was measured with a mercury manometer connected to the left common carotid artery. Left renal venous pressure and mean arterial blood pressure were recorded every 5 minutes.

The following clearance techniques were used. The creatinine clearance ( $C_{Cr}$ ) was considered equivalent to the glomerular filtration rate and the clearance of para-

aminohippurate ( $C_{PAH}$ ) was considered an adequate estimate of renal plasma flow. Water excretion was recorded in some experiments as the percentage of filtrate excreted as urine, i.e. the urine flow divided by the filtration rate ( $V/C_{Cr}$ ). Sodium excretion has been treated similarly, the sodium clearance divided by the creatinine clearance ( $C_{Na}/C_{Cr}$ ) giving the percentage of filtered sodium excreted in the urine. Therefore  $1-C_{Na}/C_{Cr}$  is the percentage of filtered sodium reabsorbed. Glucose Tm( $Tm_G$ ) and diodrast Tm( $Tm_D$ ) were measured in 2 dogs to evaluate tubular function.

With few exceptions each dog received during the operative procedure an amount of 1.5 or 2.0 per cent saline solution equal to 5 per cent of its body weight. This infusion was given in about 30 minutes to one hour through a cannula inserted into the right femoral vein and was followed by the administration of appropriate doses of creatinine, para-amino-hippurate (PAH), glucose or diodrast to attain blood concentrations within a suitable range, viz. 20 to 40 mg. per cent, 0.8 to 2.5 mg. per cent, 450 to 950 mg. per cent and 20 to 50 mg. per cent, respectively. These concentrations were maintained at a relatively stable level by the administration of a sustaining infusion at a constant rate of 2.7 to 3.0 cc. per minute. The amounts of the drugs for the initial dose and the infusion were calculated on the basis of the weight of each dog.<sup>4</sup> Fifteen or 20 minutes after starting the sustaining infusion or after the last surgical manipulation, depending on whichever came later, the collection of urine samples was begun. Eight to 15 cc. of blood were withdrawn every 15 to 30 minutes through a retention needle inserted into the right femoral artery. Potassium oxalate was used as an anticoagulant. Blood samples were centrifuged and the plasma separated. Creatinine, PAH, glucose and diodrast were determined in plasma filtrates made with  $CdSO_4$  according to the method of Fujita and Iwatake (19). Urine samples were collected directly in graduated cylinders and prepared for analysis by suitable dilutions. Creatinine was determined by a modification of Bonsnes's method (20), PAH by the method described by Goldring and Chasis (21), glucose by the Nelson-Somogyi method (22) and diodrast by Alpert's method (23), modified in this laboratory by Fithian and Baker (24). Sodium analyses were carried out on an internal standard type flame photometer.

#### RESULTS

The 13 dogs<sup>5</sup> in which experimental procedures were satisfactory have been divided into 4 groups. In *Group I* were those dogs in which venous pressure was

<sup>4</sup> In *dog 26* no sodium was administered; both initial and sustaining infusions consisted of a solution of 10% dextrose in water. In *dog 23* the amounts of water and sodium administered were limited. The initial infusion was an amount of 0.85% saline solution equal to 3% of the body weight and the sustaining infusion, likewise isotonic saline solution, was given at a rate of 2.6 cc/min. In *dogs 22* and *27* glucose Tm and in *dog 27* diodrast Tm were determined. These dogs received initially an amount of isotonic saline solution equal to 8 to 10% of body weight. The sustaining infusion of isotonic saline solution, which contained 15% glucose and 1.8% diodrast, was administered at a rate of 5 to 6 cc/min.

<sup>5</sup> Clearance values were obtained on 22 dogs but the results in 7 were not considered valid because the filtration rate fell off more than 25% during the course of the experiment. The mean arterial blood pressure also gradually declined, usually to below 100 mm. Hg. Glucose Tm was determined in 2 of the 15 satisfactory experiments. The control values for  $C_{PAH}$ ,  $C_{Cr}$  and filtration fraction per square meter of surface area were somewhat higher than those obtained by Selkurt (25).

raised to between 100 to 200 mm. saline, in *Group II* those in which venous pressure was raised to between 200 to 300 mm. saline and so on. With three exceptions the initial pressure was  $100 \pm 20$  mm. saline. It has been assumed that the control pressure in the right renal vein was approximately the same as the control pressure in the left. The classification of the experiments into these 4 groups has been maintained throughout the presentation of the data in figures 1 through 6. In these figures the abscissa is divided into three sections. The first comprises the three control periods of urine collection at the initial venous pressure of 80 to 120 mm. saline. The second section contains five segments, each representing a different increment in venous pressure. Each of the five segments is subdivided into the three separate, successive periods of urine collection obtained while the venous pressure was elevated. Hence, it is possible to illustrate the effect on renal function of the duration of the increase in venous pressure as well as the effect of the height of the venous pressure. The third, or recovery section, comprises the three periods of urine collection following return of the left renal vein pressure to its control value. The results observed in any one dog will fall into the control section, one of the five segments of the increased venous pressure section, and into the recovery section. On the ordinate in all six figures are plotted the values which represent the function of the left kidney as compared to that of the right. The values representing the relative function of the left kidney are not the actual value differences between the two kidneys but are the percentages that the function in the left kidney differed from the same function in the right. For example, if the plasma flow through the left kidney was 105 cc/min. and that through the right kidney was 100 cc/min., the value plotted on the ordinate would be  $\frac{105}{100} \times 100$  or 105 per cent. The horizontal dotted lines in the figures indicate the values  $\pm$  twice the standard deviation of the mean of the control values. It is to be noted that during the three control periods of urine collection the function of the left kidney did not deviate from that of the right by more than  $\pm 10$  per cent, i.e. the function of the left was 90 to 110 per cent of that of the right. For this reason it has been assumed that any deviation of much more than 10 per cent during a period of increased venous pressure may be considered a significant deviation and attributable to the increased pressure in the left renal vein.

*Effect of Increased Renal Venous Pressure on Renal Plasma Flow, Glomerular Filtration Rate and Filtration Fraction.* Para-aminohippurate clearance ( $C_{PAH}$ ) was measured in 10 dogs (fig. 1). Of these, 6 dogs showed no significant alteration of renal plasma flow through the left kidney despite elevation of the venous pressure up to 350 mm. saline for as long as 52 minutes. In 2 dogs  $C_{PAH}$  fell initially in the left kidney but only to a minimally significant extent and rose to control values again before the venous pressure was brought back to the control level. In 2 dogs (6 and 10), when the venous pressure was raised to 550 mm. saline, there was no initial fall in  $C_{PAH}$  but, after 15 to 20 minutes at that pressure, there was a significant drop in left renal plasma flow, 22 per cent and 66 per cent in dogs 10 and 9 respectively.

Glomerular filtration rate ( $C_{Cr}$ ) was likewise essentially uninfluenced by elevation of the renal venous pressure up to 350 mm. saline (fig. 2).  $C_{Cr}$  fell slightly in

on similarly anesthetized dogs. Average figures with standard deviations were  $C_{PAH} 173 \pm 40$  cc/min.,  $C_{Cr} 54 \pm 10$  cc/min. and F.F.  $0.35 \pm 0.06$ . None of the clearances for the experiments accepted as valid fell outside twice the standard deviation of the mean.

the left kidney in one dog (No. 5) in which the pressure in the left renal vein was maintained at 350 mm. saline for 52 minutes. There were significant decreases in glomerular filtration rate of 18 and 52 per cent respectively in dogs 10 and 9 in which the venous pressure was raised to 550 mm. saline.

The filtration fraction (F.F.) was not consistently altered by the elevation of the renal venous pressure until the venous pressure was raised to as high as 550 mm. saline. It was increased about 15 per cent in dog 9 and 5 per cent in dog 10. It is possible that a greater elevation of filtration fraction was masked by so-called 'vicarious' extraction of PAH by functioning nephrons from blood which had passed through the glomeruli of non-functioning nephrons.

*Effect of Increased Renal Venous Pressure on Water and Sodium Excretion.* In contrast to the lack of effect of moderate increases in venous pressure on renal plasma flow and filtration rate were the marked decreases in sodium and water excretion produced by only moderate elevation of renal venous pressure. This effect on urine flow ( $V$ , fig. 3) and sodium excretion ( $UV_{Na}$ , fig. 4) was observed in one of two dogs, 23 and 25, when the venous pressure was raised to as little as 160 mm. saline. Further increments in venous pressure in other dogs decreased the excretion of water and sodium still more. In general the reduction of urine flow and sodium excretion tended to vary directly with the height of the venous pressure. Dog 13 constituted the only exception, the absence of significant effect on water and sodium excretion possibly being related to lack of parallel function between the two kidneys.

The depression of water and sodium output was either maintained throughout the periods of increased venous pressure or water and sodium loss became more marked with time, i.e. urine flow and sodium excretion from the left kidney continued to decrease throughout the three successive periods of increased venous pressure. Dog 24 was the only exception in this respect and for no apparent reason.

Following release of the clamp and return of the venous pressure to control levels the functions of the left kidney invariably returned toward control values although usually incompletely in the 10 to 20 minutes allotted. In no experiment was there a further decline in water or sodium excretion.

The changes in the percentages of filtered water and sodium excreted ( $V/C_{Cr}$ , fig. 5, and  $C_{Na}/C_{Cr}$ , fig. 6, respectively) produced by moderate increases in renal venous pressure are further evidence that the reduction in water and sodium excretion occurred independently of minor fluctuations in the filtration rate. The marked decreases in the percentages of filtered water and sodium excreted which occurred when the venous pressure was raised to above 200 mm. saline also indicate that increased percentages of filtered water and sodium were reabsorbed.

The complete data of a typical experiment are given in table 1. Elevation of the pressure in the left renal vein to 340 mm. saline caused no significant reduction in either renal plasma flow or glomerular filtration rate in the left kidney but the urine flow and the amount of sodium excreted fell markedly during the 27 minutes that the renal venous pressure was kept elevated. The figure for percentage of filtered sodium reabsorbed is considered accurate to within  $\pm 1$  per cent. Hence, during the time when venous pressure was elevated the difference of 3 per cent between the values for the two kidneys in the third period indicates that there was a significant increase in the rate of sodium reabsorption. Since, in this experiment, there was no

correlation between the percentage of sodium load reabsorbed and the magnitude of the load, it is concluded that the relative increase of 3 per cent in the left kidney was a result of the heightened pressure in the left renal vein.

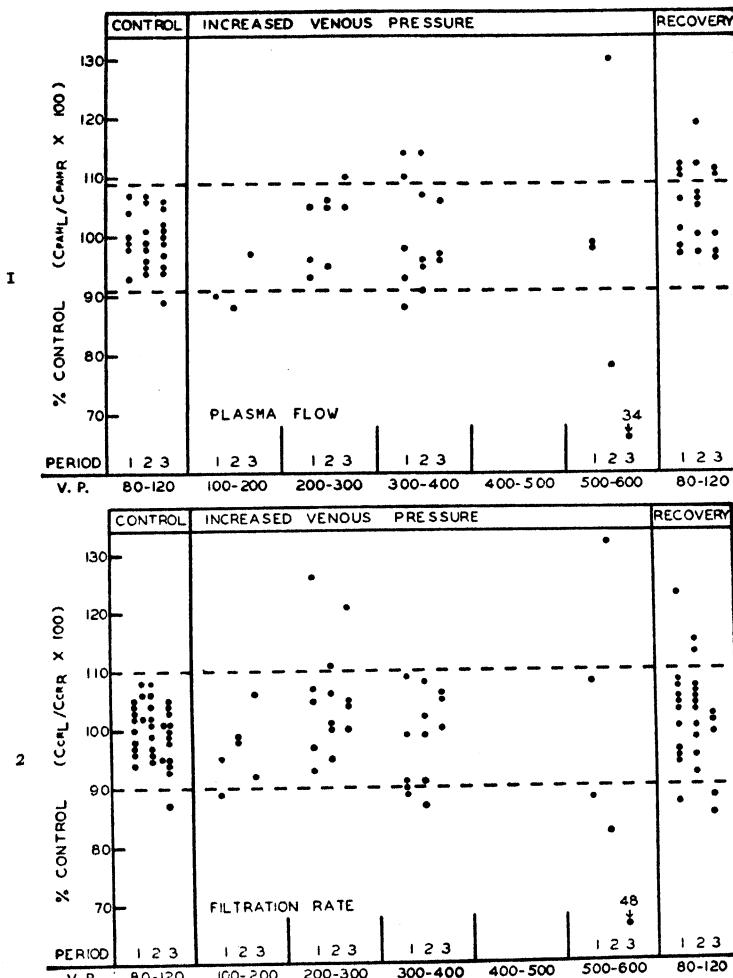


Fig. 1. EFFECT OF INCREASED VENOUS PRESSURE ON RENAL PLASMA FLOW. No significant depression of plasma flow occurred until the venous pressure was elevated to between 500-600 mm. saline.

Fig. 2. EFFECT OF INCREASED VENOUS PRESSURE ON GLOMERULAR FILTRATION RATE. No significant depression occurred with venous pressure elevated up to 350 mm. saline pressure. Reduction in filtration rate did occur in 2 dogs at 550 mm. saline pressure.

The sodium load in 11 of the 13 experiments was raised above normal by the infusion of 1.5 or 2.0 per cent saline solution. In dog 24 the infusion was isotonic saline solution and in one other (dog 26) it was 10 per cent dextrose in water. In

these 2 dogs in which the sodium load was normal and below normal respectively (serum sodium was 127 mEq/l. in dog 26) the percentage changes in decreased water

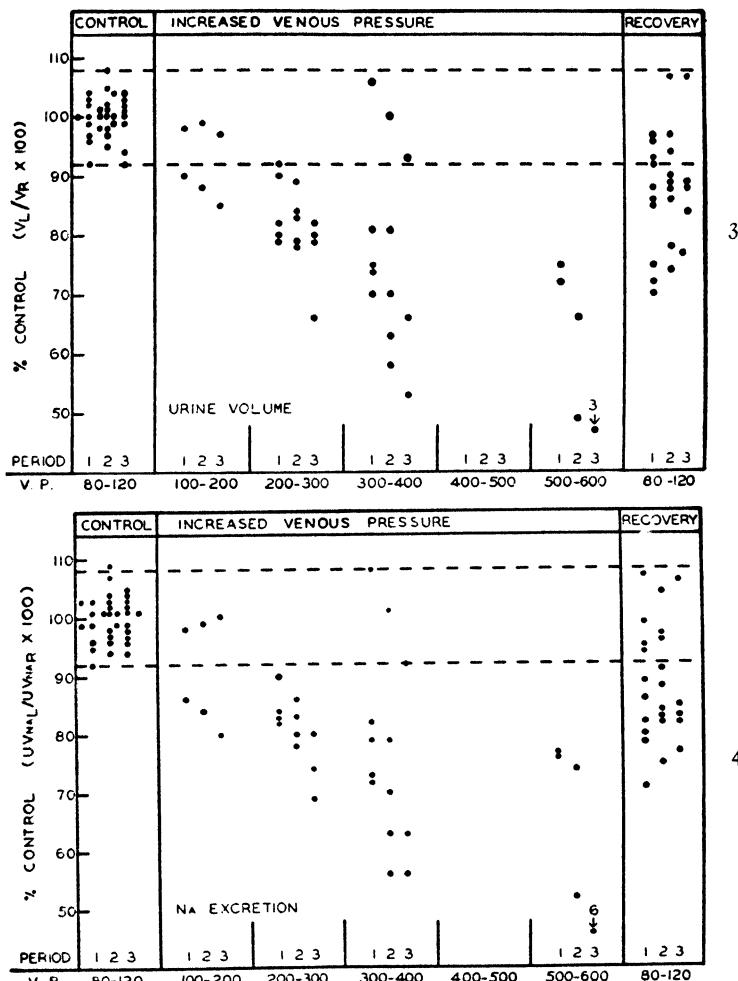


Fig. 3. EFFECT OF INCREASED VENOUS PRESSURE ON URINE FLOW. Depression of urine flow occurred in one of 2 dogs at 160 mm. saline pressure. Greater increments of venous pressure in other dogs further depressed urine flow.

Fig. 4. EFFECT OF INCREASED VENOUS PRESSURE ON TOTAL SODIUM EXCRETED. The results are comparable to those obtained on urine flow.

and sodium excreted were comparable to those observed in the other dogs. In dog 26 the actual decrease in sodium excretion was, of course, much smaller.

Glucose Tm was measured in 2 dogs and diodrast Tm simultaneously in one of these (table 2). In both dogs there was a decrease in urine flow during elevation of the venous pressure without any significant change in glucose Tm or diodrast Tm.

## DISCUSSION

By means of the techniques employed, it was demonstrated that elevation of the renal venous pressure from a mean normal of 100 mm. saline up to 340 mm. saline had no significant effect on renal plasma flow or glomerular filtration rate. Mean arterial blood pressure also remained essentially the same throughout the course of each study. Hence, elevation of the pressure in the left renal vein decreased the

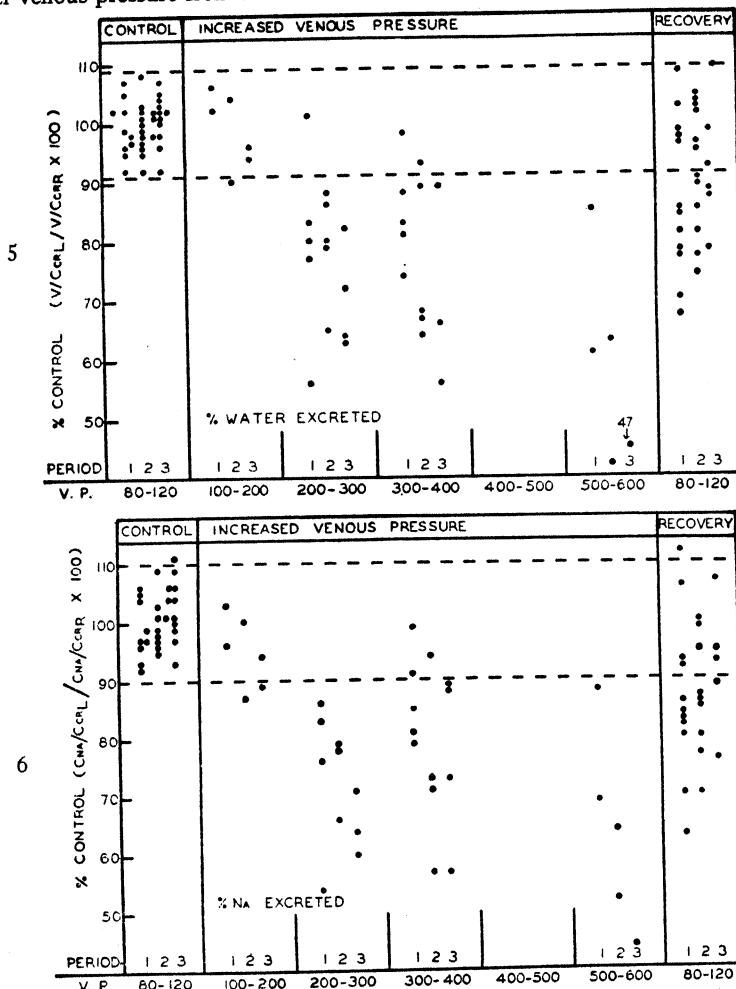


Fig. 5. EFFECT OF INCREASED VENOUS PRESSURE ON PERCENTAGE OF FILTRATE EXCRETED AS URINE, i.e., the percentage of filtered water excreted. At venous pressures above 200 mm. saline, there was depression of the percentage of filtered water excreted. This indicates that an increased percentage of filtered water was reabsorbed.

Fig. 6. EFFECT OF INCREASED VENOUS PRESSURE ON PERCENTAGE OF FILTRATED SODIUM EXCRETED. The results are comparable to those obtained on the percentage of filtered water excreted. This indicates that an increased percentage of filtered sodium was reabsorbed.

had no significant effect on renal plasma flow or glomerular filtration rate. Mean arterial blood pressure also remained essentially the same throughout the course of each study. Hence, elevation of the pressure in the left renal vein decreased the

pressure gradient from renal artery to vein. The constant plasma flow in the presence of a decreased pressure gradient implies a fall in the resistance within the renal circuit. Since there was no change in filtration rate or filtration fraction, the fall in resistance probably occurred distal to the glomerulus in the efferent arteriole and/or the peritubular capillary bed.

TABLE I. RESULTS OBTAINED IN DOG 12, 20.5 KG. FEMALE

PERIOD	LEFT RENAL V. P.	V	CREATININE						PAH						F.F.	
			U		P	UV/P		U		P	UV/P					
			R	L		R	L	R	L		R	L	R	L		
		mm. sal.	cc/min.	mg. %	mg. %	cc/min.	mg. %	mg. %	cc/min.						mm. sal.	
I	105		1.70	1.80	856	850	27.3	53	56	121	118	1.57	131	134	.41	.42
2	"		2.26	2.78	592	533	27.7	48	54	90	80	1.52	133	146	.36	.37
3	"		2.96	3.35	475	416	28.4	50	49	76	66	1.46	155	151	.32	.33
4	340		3.80	3.10	378	422	28.6	50	47	59	64	1.43	156	144	.32	.33
5	"		3.99	2.86	353	472	28.7	49	47	53	69	1.42	147	140	.33	.34
6	"		3.38	2.38	350	550	28.8	41	46	54	78	1.41	130	132	.32	.35
7	130		3.56	3.00	384	453	28.9	47	47	54	66	1.42	136	139	.35	.34
8	"		3.12	2.88	432	466	29.0	47	46	59	65	1.42	131	131	.36	.35
9	"		2.27	2.30	538	571	29.6	41	44	79	81	1.48	122	126	.34	.35
PERIOD	ELAPSED TIME	UNa		PNa		UV/P		CNa/Ccr <sup>1</sup>		PNa × Ccr <sup>2</sup>		UVNa <sup>3</sup>		1 - CNa/Ccr <sup>4</sup>		M.EAN ART. PRESS.
		R	L	min.	meEq/l.	meEq/l.	cc/min.	R	L	R	L	meEq/min.	meEq/min.	%	mm. Hg	
I	0-10	252	252	154	2.79	2.05	.052	.053		8200	8600	430	450	95	95	145
2	10-18	234	234	154	3.44	4.23	.071	.079		7400	8200	530	650	93	92	"
3	18-29	201	201	154	3.87	4.38	.078	.089		7700	7600	600	670	92	91	"
4	29-39	177	185	154	4.36	3.82	.087	.081		7700	7300	670	590	91	92	145
5	39-47	167	170	155	4.30	3.31	.088	.070		7600	7300	670	510	91	93	"
6	47-56	166	182	155	3.62	2.79	.088	.061		6400	7100	560	430	91	94	"
7	56-66	166	176	155	3.81	3.40	.080	.072		7300	7300	590	530	92	93	140
8	66-74	165	168	155	3.31	3.11	.071	.067		7200	7200	520	480	93	93	"
9	74-80	165	169	156	2.41	2.50	.058	.056		6400	6900	380	390	94	94	"

<sup>1</sup> Percentage of filtered sodium excreted. <sup>2</sup> Filtered sodium (sodium load). Uncorrected for Donnan equilibrium. <sup>3</sup> Excreted sodium. <sup>4</sup> Percentage of filtered sodium reabsorbed. V represents urine flow; U represents urine concentration; P represents plasma concentration 2 minutes prior to the midpoint of the urine collection period; UV/P represents clearance. F.F. is filtration fraction.

When venous pressure was raised to 550 mm. saline the renal plasma flow and glomerular filtration rate were reduced. In these experiments the decreased resistance in the post-glomerular circuit was presumably insufficient to compensate for the fall in pressure gradient and the renal plasma flow decreased. The increased filtration fraction observed in these experiments indicated that a significant degree

of back pressure extended proximal to the efferent arteriole and thereby increased effective filtration pressure. Regardless of the exact quantitative hemodynamic relationships involved, it seems most probable that significant peritubular capillary congestion did occur.

The effect observed on water and sodium excretion is in contrast to that on plasma flow and filtration rate. In one dog elevation of the renal venous pressure to only 160 mm. saline resulted in an appreciable decrease in urine flow and sodium excretion. With more marked increases in renal venous pressure the effect was more pronounced. The decreased excretion of water and sodium without concomitant reduction in filtration rate indicates an increased rate of water and sodium reabsorp-

TABLE 2. EFFECT OF INCREASED VENOUS PRESSURE (V.P.) ON URINE FLOW (V), FILTRATION RATE ( $C_{Cr}$ ), GLUCOSE Tm ( $Tm_G$ ), AND DIODRAST Tm ( $Tm_D$ )

DOG	PERIOD	LEFT RENAL V.P. mm. sal.	V		$C_{Cr}$		$Tm_G$		$Tm_D$	
			R	L	R	L	R	L	R	L
27	1	75	6.51	6.81	49	48	162	152	7.0	7.3
	2	75	5.98	6.30	47	49	145	152	7.2	6.6
	3	75	5.43	5.63	45	47	134	142	7.4	7.5
	4	165	4.48	4.63	48	46	148	140	7.1	7.3
	5	165	4.33	4.38	48	47	145	136	7.4	7.8
	6	205	4.30	4.10	46	47	137	144	7.5	7.8
	7	70	3.58	3.72	45	47	142	143	8.1	7.7
	8	70	3.73	3.83	47	44	147	132	7.5	7.7
22	1	105	4.35	4.56	56	65	139	179		
	2	105	4.44	4.68	56	63	152	165		
	3	105	4.09	4.38	56	63	145	136		
	4	215	3.66	3.55	55	61	142	175		
	5	215	3.20	3.16	53	55	124	133		
	6	105	2.75	2.91	50	53	122	129		
	7	105	2.71	2.88	52	52	136	128		

tion. The minor fluctuations encountered in measured filtration rate may be the result of certain technical shortcomings and/or possibly variability in the number of functioning nephrons. To minimize the influence of these minor fluctuations on the observed changes in sodium excretion the sodium to creatinine clearance ratio has been calculated for each urine-collection period. Water excretion has been handled similarly. The values for these ratios ( $V/C_{Cr}$  and  $C_{Na}/C_{Cr}$ , the percentages of filtered water and sodium excreted respectively) were also decreased by increased venous pressure, thus indicating again an increase in the percentages of filtered water and sodium reabsorbed. It might be worthwhile to point out that although small percentile changes in filtration rate may cause greater percentile

changes in  $UV_{Na}$  and hence influence  $C_{Na}/C_{Cr}$ , this explanation cannot be invoked to invalidate the interpretation of the results. It is apparent from figure 2 that during the periods of increased venous pressure the filtration rate in the left kidney was greater than that in the right as often as it was smaller. Nevertheless, with one exception, there always was observed a decrease in urine volume and excreted sodium. It seems highly unlikely that the consistent decreases in urine volume and excreted sodium in the left kidney can be ascribed to a decrease in the glomerular filtration rate.

In general, the diminution in sodium and water excreted was progressive with time and was related to the height to which the pressure in the renal vein was raised. In only one dog did a return toward the control values occur prior to returning the venous pressure to the control level. In no experiment was renal venous pressure kept up for more than 52 minutes. Therefore, it is impossible to say whether or not chronic elevation of the venous pressure would lead to chronic retention of water and sodium and other possible secondary effects such as decreased renal plasma flow and glomerular filtration rate. But, regardless of what happens to renal plasma flow and glomerular filtration rate and regardless of the mechanism involved in the decreased excretion of sodium, the important fact remains that under the stated conditions the increased venous pressure did cause a significant retention of water and sodium.

The mechanism involved in the increased reabsorption of water and sodium as a result of increased renal venous pressure is not apparent. The effect was immediate and local, i.e. limited to the kidney in which the venous pressure was raised. This seems to eliminate as a cause any humoral or central reflex mechanism. It seems probable that the effect was mechanical rather than due to any specific alteration of the intrinsic physiology of the tubular cells. The lack of change in glucose Tm or diodrast Tm tends to support this conclusion. There is no definitive evidence to indicate whether the increased reabsorption of water was independent of or dependent on the increased reabsorption of sodium or vice versa. It is possible that the peritubular capillary congestion was responsible for the increased water and sodium reabsorption either by partially obstructing the flow of urine through the tubules or by increasing the time and capillary surface area available for transfer of water and sodium.

It is impossible to say whether or not the results obtained in acute experiments on anesthetized dogs have any bearing on the pathogenesis of edema in clinical cardiac failure. Certainly the increase in renal venous pressure necessary in our studies to produce a decrease in sodium and water excretion is well within the range of venous pressures found in frank cardiac failure or even before frank cardiac failure, during exertion. Hence, the rise of venous pressure occurring in cardiac failure may very well be a factor contributing to the formation of edema in cardiac failure. The possible rôle played by the increase in venous pressure in the formation of cardiac edema does not detract from the rôle played by the reduction in renal blood flow and filtration rate in the excretion of water and sodium. However, the factor of decreased renal blood flow and glomerular filtration rate (10, 11) has been claimed to be responsible for the decreased excretion of sodium and water in cardiac failure, although the retention of sodium and water can be explained in our experiments on the basis of increased renal venous pressure alone. In terms of the Starling law of the heart,

the reduction in cardiac output occurs later in the development of cardiac failure than the increase in right ventricular pressure. In other words, the cardiac output is maintained at a normal level, everything else being equal, because the heart contains more residual blood at the end of diastole. One may wonder if the increase in the intraventricular pressure does not lead ultimately to an increase of the renal venous pressure before a decrease in cardiac output occurs.

In short, it is probable that reduction in renal blood flow and filtration rate as well as the increase in renal venous pressure plays a rôle in the formation of the edema of cardiac failure. However, the causal and temporal relationship of these two factors in the formation of cardiac edema and their relative importance remain uncertain.

#### SUMMARY

The effect of increased renal venous pressure on renal function was studied in anesthetized dogs by means of clearance techniques. Renal function was measured separately and simultaneously in the two kidneys but the pressure was raised in the left renal vein only, by means of a specially designed clamp. Venous pressure was measured with a saline manometer through a venous catheter which had been passed into the left renal vein. Control values for the functions of the left kidney were obtained before and after elevation of venous pressure and were comparable to controls obtained from the right kidney. Under the stated conditions moderate elevation of the left renal venous pressure up to 350 mm. saline caused in that kidney a significant decrease in water and sodium excretion without any change in the renal plasma flow, glomerular filtration rate, glucose Tm or diodrast Tm. The reduction in water and sodium excretion was due to an increase in the reabsorption rate of these substances by the renal tubule cells. This effect also occurred when the sodium load was low, for example in one experiment in which the serum sodium was 127 MEq/l. Greater elevation of venous pressure to 550 mm. saline decreased renal blood flow and filtration rate, but the results were not sufficient to state whether filtration fraction was significantly altered.

The mechanism for the increased reabsorption of sodium and water ascribed to increased venous pressure was not obvious. It was local, i.e. confined to the kidney in which the venous pressure was raised and consequently was not related to release of pituitary, adrenal and hepatic hormones. It probably was mechanical rather than the result of any alteration in specific metabolic processes of the tubular cells since there were no associated changes in glucose Tm or diodrast Tm. Some of the implications of these results with respect to the pathogenesis of edema in cardiac failure have been discussed.

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# REABSORPTION OF CREATINE AND GUANIDOACETIC ACID BY THE RENAL TUBULES<sup>1</sup>

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IT HAS been well established that creatine is formed in experimental animals (1, 2) and in human beings (3) by the methylation of guanidoacetic acid (glycocyamine). *In vitro* tissue experiments have indicated that the kidney is the predominant site of synthesis of guanidoacetic acid from arginine and glycine (4). Since its detection in normal human urine by Weber in 1935 (5) guanidoacetic acid has been consistently found in the urine of adults and children in quantities ranging from 30 to 150 mg. per day. This is in contrast to creatine, which is normally not detectable in the urine of normal adult males or in the majority of females (6). Since the liver is the most likely site of the methylation of guanidoacetic acid, the tolerance to injected and ingested guanidoacetic acid has been studied in liver disease (7-9). On the basis of preliminary observations it appeared that the ratio of endogenous urinary guanidoacetic acid to total creatinine was elevated in cirrhosis (8). During recent studies on creatine metabolism (3) it was found that the administration of creatine orally or intravenously produced a striking increase in guanidoacetic acid excretion. This suggested either an inhibition of guanidoacetic acid methylation under such conditions or a competition by guanidoacetic acid and creatine for renal tubular reabsorption. Recent studies have made the former possibility less likely (25, 10). Two conditions would have to be satisfied in order to substantiate the latter hypothesis: 1) a fall in the serum concentration of endogenous guanidoacetic acid during the administration of creatine, and 2) the demonstration by clearance techniques that the renal tubular reabsorption of guanidoacetic acid was blocked. Both of these criteria were fulfilled in the following studies.

## EXPERIMENTAL

*Analytical Methods.* All analyses were in duplicate. Guanidoacetic acid (GAA) was determined by the method of Hoberman (11). Arginase was prepared and activated with cobalt according to the method of Hunter (12). Portions were stored at -40° C and thawed shortly before use, since a lyophilized preparation was found to lose potency rapidly. Four standards were run with each set of determinations. Since the Sakaguchi color from serum dialysates remains constant while that from standard solutions fades on warming to room temperature, readings were made at a constant interval after the addition of hypobromite. The reliability of the method was increased by sealing the margins of the dialysing membrane with a plastic cement and by extracting each 5 cc. of dialysate with 0.5 cc. of water-saturated chloroform to remove any traces of protein. Creatine produces a faint Sakaguchi reaction. Twenty mg. of creatine gave color equivalent to 1 mg. of GAA in all dilutions, and corrections were made on this basis. All urines were extracted with chloroform and in certain instances, noted in the table, were incubated with arginase. The method was found to be accurate to 5 per cent.

Received for publication December 28, 1948.

<sup>1</sup> Aided by a grant from the Fluid Research Fund of Yale University School of Medicine.

Creatinine and creatine were determined by the method of Peters (13). To minimize the error introduced by the progressive darkening of the Jaffe color from serum filtrates, readings were made at a fixed 20-minute interval after addition of alkaline picrate. Since color from both preformed and total creatinine increases proportionally, the error in creatine determination was minimal from this source. Under the conditions of the present method and of the original Folin method, GAA is partially converted to glycocyamidine, which also give the Jaffe color. When GAA is added to standard solutions or to urine in the present method, 6 to 7 mg. of GAA are found to be equivalent in color production to one mg. of creatine. The exact ratio varies with the duration of autoclaving. In serum filtrates, however, the color production from GAA varies with the final acidity of the particular filtrate. It was found that this variation could be avoided by adding 0.5 cc. of 1N HCl to 32 cc. of the serum filtrates. In order to control each set of determinations, 7.5, 15, and 30 gamma of GAA were added before autoclaving to the filtrates from a urine and a serum sample obtained at the start of the experiment. In this manner a correction curve was established. Thymol was added to all urines as preservative. Analyses were done with a minimum of delay to avoid spontaneous conversion of creatine to creatinine and any bacterial destruction of creatinine. The creatinine determination is accurate to 5 per cent or better, while the error in determining creatine is somewhat larger, since it is estimated by difference.

Mannitol was determined by the method of Elkinton (14), the standard error of which is  $\pm 1.2$  mg. per cent for serum and  $\pm 88$  mg. per cent for urine. Fresh yeast was used in each set of determinations.

*Plan of Experiments.* The authors served as subjects. Experiments were run 4 to 5 hours following a creatine-free meal. 250 to 350 cc. of water per hour were ingested for 5 hours prior to and during the period of observation to obtain a urine flow of 6 to 10 cc. per minute. The subjects were not catheterized, but urine collections were delayed until a volume of over 350 cc. was obtained to minimize the effect of residual urine. The subject was kept reclining with feet horizontal, and one hour was allowed for glomerular filtration to reach a constant rate. 100 cc. of 25 per cent mannitol solution was then injected, and 35 to 40 minutes later, after equilibrium was established, the glomerular filtration rate was measured. Another intravenous injection of mannitol was given before each subsequent period to obtain comparable serum levels. During the majority of these periods, as indicated in table 1, constant infusions of GAA and creatine in physiological saline were given. The rate of infusion was precisely controlled at 480 cc. per hour by means of a tunnel clamp. These infusions were preceded by suitable booster doses given at the time of the mannitol injections. Venous blood samples were drawn with a Cournand needle at the beginning and end of each urine collection period. Before veni-puncture the forearm and hand were immersed in hot water to avoid the necessity for arterial punctures.

*Calculations.* Glomerular filtration rates of both mannitol and endogenous creatinine were calculated in the conventional manner from the mathematical mean of the serum concentrations bracketing each period and the urinary excretion. Values were not corrected for surface area. The amount of creatine or of GAA filtered has been calculated as the product of the mean concentration in mg. per cc. of serum multiplied by the mannitol clearance in cc. per hour. The amount reabsorbed by the tubules is the differences between this value and the excretion per hour.

## RESULTS

Analytical data are given in table 1.

*Experiments 1 and 2 show the effect on normal GAA excretion of increasing the*

amount of creatine filtered from 20 mg. per hour to 123 and 54 mg. respectively. Even at rates of filtration only slightly higher than that necessary to produce creatinuria the excretion of GAA increased and its tubular reabsorption decreased. At the same time the serum level of GAA fell 0.02 mg. per cent. This fall is consistent

TABLE I. EFFECT OF INFUSIONS OF CREATINE AND GUANIDOACETIC ACID ON GUANIDOACETIC ACID TUBULAR REABSORPTION

EXPERIMENT	SUBJECT	INJECTION		GLOMERULAR FILTRATION RATE		GUANIDOACETIC ACID <sup>1</sup>				CREATINE <sup>1</sup>			
		Cr. <sup>2</sup>	GAA <sup>2</sup>	Mannitol	Creatinine	Serum conc.	Filtered	Excreted	Reabsorbed	Serum conc.	Filtered	Excreted	Reabsorbed
<i>1</i>	<i>EAHS</i> wt., 63.5 kg. ht., 72.5 in.	0	0	107	103	0.23	14.8	3.5	11.3	0.32	20.5	0	20.5
		0.25	0	100	106	0.21	12.6	6.9 <sup>4</sup>	5.7	2.05	123.0	29.6	93.4
<i>2</i>	<i>DWS</i> wt., 66.0 kg. ht., 72.0 in.	0	0	91	83	0.20	11.0	2.4	8.6	0.38	20.7	0	20.7
		0.25	0	78	95	0.18	8.4	3.6	4.9	1.15	54.0	5.8	48.2
<i>3</i>	<i>EAHS</i>	0	0	119	121	0.24	17.2	4.7	12.5	0.45	32.0	0	32.0
		0	0.25	100	119	0.96	57.6	27.8	29.8	0.41	24.6	0	24.6
		0.5	0.25	96	131	0.53	30.5	27.1	3.4	0.90	52.0	5.4	46.6
<i>4</i>	<i>EAHS</i>	0.075	0	117	132	0.22	15.4	3.9	11.6	0.60	42.0	0	42.0
		0.075	0.15	91	112	0.83	45.2	21.9	23.3	0.57	31.0	0	31.0
<i>5</i>	<i>DWS</i>	0.25	0	101	93	0.16	9.7	3.3	6.4	1.44	88.6	11.7	76.9
		0.25	0.275	99	92	0.98	58.2	61.0	-2.8 <sup>3</sup>	1.42	84.4	15.6	68.8
<i>6</i>	<i>DWS</i>	0.25	0	103	85	0.23	14.2	3.1	11.1	1.22	75.4	10.3	65.1
		0.25	0.75	105	95	2.61	165.0	158.0 <sup>4</sup>	7.0	1.16	73.1	14.0	59.1
<i>7</i>	<i>EAHS</i>	0	0.5	121	101	1.95	141.0	91.0	50.0				
		0	1.5	110	107	6.71	442.0	239.0	203.0				

<sup>1</sup> Guanidoacetic acid and creatine filtration rates were calculated on the basis of the mannitol clearances. The results would not vary significantly were creatinine clearances used.

<sup>2</sup> Cr = creatine; GAA = guanidoacetic acid. Infusions (preceded by boosters) were given at a constant rate in the amounts indicated, yielding constant serum levels.

<sup>3</sup> This negative value is considered within the error of the methods, and is not interpreted as indicating tubular secretion of GAA.

<sup>4</sup> No arginine could be detected on incubation with arginase.

in magnitude and direction with the reduction predicted from the increased excretion, if it is assumed that GAA is distributed uniformly throughout the body water.

In experiment 3 GAA was first infused in an amount sufficient to double its tubular reabsorption, and subsequently creatine was administered. When the creatine load was thus increased, the reabsorption of GAA fell to half its initial value. *Experi-*

ment 4 serves as a control observation illustrating that when no creatinuria existed an increase in GAA filtered still increased its reabsorption.

In experiments 5 and 6 GAA was injected in an effort to inhibit the reabsorption of creatine by the tubules. There was a moderate creatinuria of 14 to 15.6 mg. per hour in the initial periods, following which the quantity of GAA filtered was raised four-fold and ten-fold (from 9.7 mg/hour to 58.2, and from 14.2 to 165 mg/hour respectively). A moderate increase in creatinuria was found, together with a reduction in tubular reabsorption, but since tubular reabsorption of GAA was also reduced, the results are regarded as inconclusive.

In experiment 7 the reabsorption of GAA at a relatively high serum level (6.7 mg. %) increased roughly in proportion to the amount filtered.

TABLE 2. URINARY EXCRETION OF GUANIDOACETIC ACID AND CREATINE IN NORMAL SUBJECTS AND PATIENTS WITH VARIOUS DISEASES

SUBJECT	SEX	DIAGNOSIS	GUANIDOACETIC ACID <sup>1</sup>	CREATINE <sup>1</sup>
ES	M	Normal male	68	0
DS	M	" "	41	0
HH	M	" "	66	0
AS	M	" "	52	0
BS	M	" "	62	0
JT	M	Hepatitis	72	0
AR	M	Advanced cirrhosis	61	0
WB	M	Myotonia atrophica	32	0
DW	M	Addison's disease	55	0
MN	F	Ovarian agenesis	100	40
FS	F	Normal female	69	46
ED	M	Thyrotoxicosis	83	53
TC	M	Myotonia atrophica	31	55
PP	M	Advanced cirrhosis	192	68
CB	F	Secondary syphilis	141	129
JM	M	Myotonia atrophica	93	140
AP	F	Thyrotoxicosis	255	300
MC	F	"	145	358

<sup>1</sup> Expressed as mg/gm. of creatinine.

From the results above it would be expected that creatinuria from any cause should be accompanied by a high level of GAA excretion. In table 2 are summarized the results of analysis of urine from normal subjects and from hospital patients with various diseases. Urine from subjects with proteinuria was extracted with chloroform before analysis for GAA. In order to reduce them to comparable terms, the values for GAA and creatine are expressed as mg/gm. of creatinine excreted. The data are arranged in order of increasing creatinuria, and the expected correlation is evident from the figures. Three of the subjects, (FS, ED, and TC) do show low GAA excretion in the face of small amounts of creatine, which, however, may merely represent brief post-prandial creatinurias during the course of the urine collections. One subject with advanced cirrhosis, whose condition progressively deteriorated, shows an

excretion of GAA out of proportion to the slight creatinuria, which may indicate an impairment of methylation of GAA.

#### DISCUSSION

The relationship between the quantities of GAA and of creatine filtered to those reabsorbed is shown in figure 1, in which data from table 1 have been plotted. It is apparent from the figure that a smaller percentage of filtered GAA than of creatine is reabsorbed at all levels of filtration. This relative difference is more marked at higher levels of filtration. It has been possible by increasing creatine filtration within physiological limits to demonstrate a blocking effect upon the tubular reabsorption of GAA. It was not possible, however, to demonstrate the reverse effect, namely inhibition of creatine reabsorption by increase in GAA filtration. This is not surprising for two reasons. First, it is clear from a consideration of figure 1 that the reabsorptive mechanism shows less affinity for GAA than for creatine. Secondly, GAA

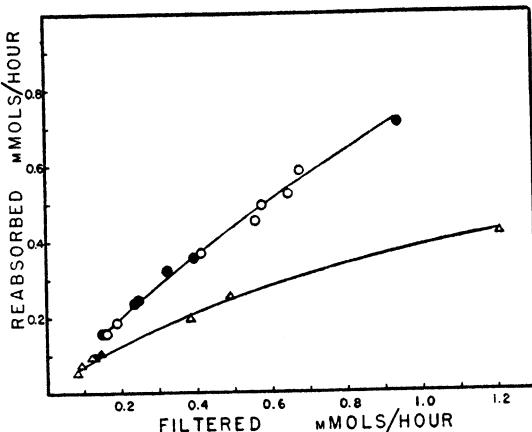


Fig. 1. RELATION BETWEEN QUANTITY FILTERED AND TUBULAR REABSORPTION of creatine and guanidoacetic acid. Circles represent creatine and triangles guanidoacetic acid. Hollow symbols are from subject *DWS* and solid from *EAHS*. The experiments were performed over a period of 3 months.

is always present in the urine while creatine is not found in detectable amounts, at least in adult males and in the majority of females, despite the fact that they are filtered in comparable amounts.

It is apparent from the figure that in the case of both substances, at all levels of filtration studied, less is reabsorbed than is filtered. Hence there is no evidence that either substance is excreted by tubular secretion. As the filtration of each substance increases, the quantity reabsorbed also increases, although at a diminished rate. In the case of creatine this is consistent with the finding of Zierler (15) that in normal subjects it has not been possible definitely to demonstrate a value for maximal tubular reabsorption of creatine even when serum levels are raised to 35 mg. per cent. The maximal tubular reabsorption of GAA, if such exists, has not been explored because of the magnification of error involved in estimating tubular reabsorption by difference at high levels of filtration. The constancy of the reabsorptive mechanism for the two substances is shown by the fact that values from both subjects fall on the same lines.

In calculating tubular reabsorption glomerular filtration rates were determined by mannitol. Evidence has recently been obtained from simultaneous determinations that mannitol clearances are from 0.87 (16) to 0.9 (17) smaller than inulin clearances. Were this substantiated, the absolute values for tubular reabsorption given in table I would be proportionally low. Since mannitol clearances are presumably proportional to actual changes in glomerular filtration rate, however, the relative changes are unaffected.

Evidence has been advanced suggesting that substances other than GAA compete with creatine for a common tubular transport system. Pitts has presented data which suggest a blocking of the tubular reabsorption of creatine by glycine (18), by alanine and to a lesser extent by glutamic acid (19). By similar methods glycine was shown to block reabsorption of arginine, but competition was not demonstrated between arginine and creatine. He has proposed a common renal mechanism for tubular reabsorption of the five substances. The results are not entirely conclusive because extremely high rates of filtration and secretion of creatine were employed. Deductions were thus based on changes that are close to the limits of the methods in the relatively small amounts of creatine reabsorbed. In addition, Beyer (20) has pointed out the drawbacks in such studies of using non-specific methods for the determination of amino acids. If the substances do share a common reabsorptive mechanism, it would be expected from the present studies that the amino acids would block GAA reabsorption. Evidence is as yet lacking on this point, except for the observation of Borsook (21) that GAA excretion increases following the ingestion of glycine or arginine or both, with a fall in place of the rise in serum GAA concentration that would be expected, if the increased excretion were solely the result of increased synthesis.

Thyroxine and desoxycorticosterone acetate have recently been shown to reduce the maximal tubular reabsorption of creatine in hypothyroid subjects (22). Since GAA is normally reabsorbed with less facility than creatine, similar changes in its excretion might be expected from these agents. Bodansky (23) found high levels of excretion of GAA in hyperthyroidism, but from the values tabulated above, it seems likely that this effect was secondary to creatinuria. That the increase in GAA excretion seen in man during ingestion of methyl testosterone does reflect increased synthesis is evident, first, from isotope studies (24) and, secondly, from the fact that the serum level of GAA rises before that of creatine (25).

The mechanism of the renal tubular reabsorption of creatine and of GAA is obscure. *In vitro* phosphorylation of added creatine with rat kidney homogenates has been demonstrated by Potter (26) and it is possible that creatine is phosphorylated by the tubular cell on reabsorption. But no such evidence exists to suggest that glycocystamine phosphate may be formed during reabsorption of guanidoacetic acid. Experiments are in progress to determine the effect of compounds other than creatine on this reabsorptive system.

In view of the magnitude of the increase in GAA excretion following the induction of a slight creatinuria, it is apparent that GAA tolerance tests must be controlled by simultaneous measurement of its renal excretion. Likewise, it is apparent that the relative or absolute level of GAA excretion cannot be taken as an index of the rate of its endogenous synthesis or of its methylation.

## SUMMARY

In normal males it was found that elevation of serum creatine within physiological limits increased the excretion of guanidoacetic acid and reduced its renal tubular reabsorption, both at endogenous and at elevated serum levels. The reverse effect could not be conclusively demonstrated. Increases in renal excretion of guanidoacetic acid may be a reflection of creatinuria rather than of change in the rate of guanidoacetic acid synthesis or methylation.

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# RÔLE OF THE KIDNEY IN PATHOGENESIS OF HYPERTENSION AS DETERMINED BY A STUDY OF THE EFFECTS OF BILATERAL NEPHRECTOMY AND OTHER EXPERIMENTAL PROCEDURES ON THE BLOOD PRESSURE OF THE DOG

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ONE of the difficulties involved in determining experimentally the exact rôle which the kidney plays in the pathogenesis of hypertension has been the impossibility of ablating this organ without introducing the fatal effects of interfering with its normal excretory function. For this reason, it has been impossible to apply the simple experimental procedure of extirpation which has aided so much in the elucidation of the function of other organs. It is, in fact, generally assumed that bilateral nephrectomy causes no change in the blood pressure. However, animals subjected to this procedure in the past have survived for such relatively short periods and were in such poor condition that any conclusion as to the effect of nephrectomy on the blood pressure based on observations of such animals is not convincing.

Indirect studies designed to elucidate this problem, such as the effect of unilateral or bilateral nephrectomy in the hypertensive animal (1) or in the parabiotic rat (2), have indicated that nephrectomy results in an elevation in blood pressure and that the presence of intact kidneys is essential for maintaining the normotensive state (3). In the present study, it has been possible to maintain bilaterally nephrectomized dogs in a state of good health for a longer period than has hitherto been possible. To determine the effect of the presence of renal tissue incapable of exerting any excretory function on the blood pressure, animals with one ureter ligated or implanted into the small intestine or abdominal vena cava and the contralateral kidney removed and those with bilaterally ligated ureters were studied. Observations on the blood pressure in these animals permit one to draw certain definite conclusions as to the rôle of the kidney in the pathogenesis of hypertension and indicate that the kidney, aside from its excretory function, is responsible for the maintenance of a normal blood-pressure level.

## METHODS

Adult mongrel dogs of either sex were used throughout this study. Nephrectomy was performed under ether anesthesia either in two stages with an interval of a week or more between the two operations or at one operation. In the former case, a lumbar approach was used; in the latter, the abdominal transperitoneal route was utilized. The former procedure involves much less operative shock and is preferred if adequate facilities are available for housing the animals in the interval between the operations.

Received for publication January 25, 1949.

<sup>1</sup> This work was supported by a grant from the Life Insurance Medical Research Fund.

In another group of dogs, the right ureter was freed from its accompanying blood vessels, ligated in close proximity to its entrance into the bladder, and cut. The ligated end proximal to the bladder was buried by fine sutures into the serosal lining of the bladder. The open end was transfixated with three sutures which were then passed through an opening into the small bowel and an uretero-intestinal anastomosis performed by the usual surgical procedures. This is essentially the Coffey operation as performed on the human subject<sup>2</sup> except for the fact that the implantation is made into the small intestine from which the urine is resorbed.

In a comparable series of animals the right ureter was implanted into the abdominal vena cava and the left kidney ablated. Approach to the vena cava was either through a circular incision across the upper abdominal wall or preferably through a right rectus incision. After exposing the vena cava a segment was isolated between clamps, the ureter prepared as described in the preceding paragraph, and anastomosed to an opening made in the vena cava. After an interval of one to 3 weeks to allow union at the site of the anastomosis, the contralateral kidney was removed through a lumbar incision.

In the fourth series of animals, both ureters were doubly ligated and divided or the right ureter ligated and divided, and after a period of at least two weeks, the left kidney was ablated.

Mean blood pressures were determined by direct puncture of the femoral artery with a No. 18-gauge needle attached through a 3-way valve to a syringe and to a mercury manometer (1).

Two procedures have been utilized for abolishing uremia and prolonging life of the animals prepared as described above. Prolongation of life for several days beyond that normally observed in the absence of renal excretory function was made possible by feeding the animals a diet practically free of electrolytes. Such a diet was prepared either by dialysis of the animals' normal food or by feeding a mixture of casein with a very low sodium content<sup>3</sup> to which was added glucose and lard. Animals maintained on such diets remain in much better health following bilateral nephrectomy and survive longer than do those on a free diet. We have observed that the intensity of many of the symptoms observed in the nephrectomized animal, as well as in the human patient dying in uremia, is apparently due to the accumulation of ingested electrolytes (particularly potassium) for it is greatly mitigated if electrolytes are excluded from the diet.

The survival period of the nephrectomized dog varies usually between 2 and 7 days, depending on the original state of health of the animal, the operative procedure, conditions under which the animal is kept etc. Under the conditions of our experiments, survival extended from 2 to 5 days. However, when the animals were given a 'salt-free' diet, as described above, survival was extended to at least 5 days and usually longer. To maintain animals with no renal function beyond this time required the application of an 'artificial kidney' to remove the accumulated urea and

<sup>2</sup> We are indebted to Dr. Carl Moyer for demonstrating this operation to us and performing it on dog 73.

<sup>3</sup> Liberal supplies of this casein were supplied by Mead-Johnson and Company through the courtesy of Dr. Charles Bills.

other waste products from the body and to maintain normal water, electrolyte and acid-base equilibria.

The use of the artificial kidney has been described elsewhere (4). Its successful application made it possible to prolong the life of bilaterally nephrectomized dogs or dogs otherwise deprived of renal excretory function for periods sufficiently long to permit one to draw definite conclusions as to the rôle of the kidney in the pathogenesis of hypertension. It was necessary to apply the artificial kidney on the 5th or 6th day following exclusion of renal excretory function and at intervals of 3 or 4 days thereafter. The femoral artery was ligated and the blood, after passing through the 'artificial kidney', returned to the femoral vein. A period of dialysis of  $2\frac{1}{2}$  to 3 hours was usually required to lower the urea content of the blood from 470 to 600 to less than 100 mg. per cent.

#### RESULTS

*Effect of Bilateral Nephrectomy.* In table 1 are given the mean blood pressures of 38 dogs following bilateral nephrectomy. It is evident from the results that following removal of the kidneys there is a tendency for the blood pressure to rise. This rise is evident on the third day following nephrectomy and increases thereafter as long as the animal survives. In ten instances (*dogs 35, 39, 54, 70, 75, 76, 84, 86, 89, 93, and 96*) values of 150 mm. Hg, which is usually taken as definite evidence of hypertension in the dog, were not attained. However, even in the case of these animals there is in every case but one (*dog 35*) a tendency for the blood pressure to rise above the preoperative level. The average mean blood pressure as seen in the last line of of table 1 rises gradually over the preoperative level as survival is extended.

Microscopic study of the tissues of the nephrectomized animals revealed (5) widespread cardiac damage—subendocardial hemorrhages, myocardial necrosis etc.—which would tend to prevent a sustained maximal rise in blood pressure. This is comparable to the decline in blood pressure observed in human hypertensives suffering from cardiac failure. The changes in the heart account probably for the observed rises being less pronounced than they might otherwise be.

The general trend of the blood pressure rise in the nephrectomized dog is shown graphically in figure 1 in which data on 5 dogs are reproduced to show the gradual rise and extent of the blood pressure rise which follows nephrectomy.

*Effect of Bilateral Ureteral Ligation.* In table 2 are recorded the blood pressure responses of 12 dogs in which both ureters were ligated and divided, the kidney being left intact. This operation, in accord with the findings of earlier observers (6-10), results in a rise in blood pressure which is sometimes evident on the day following the ligation and reached its maximum (with the single exception of *dog 23*) on the fourth day, declining thereafter to the normal level. This secondary decline has been overlooked by previous workers since their animals failed to survive for a period sufficiently long for it to manifest itself.

Ligation of the ureters results in hydronephrosis and marked changes in the kidneys as well as in a period of survival which is less than that observed following nephrectomy. The observed rise in blood pressure is apparently due to the liberation of a pressor agent from the injured kidney for a similar rise is noted even when only

TABLE I. EFFECT OF BILATERAL NEPHRECTOMY ON THE MEAN BLOOD PRESSURE OF THE DOG

DOG	PREOPERA-TIVELY	DAYS FOLLOWING FINAL NEPHRECTOMY												
		1	2	3	4	5	6	7	8	9	10	14	17	
26	110		145		160									
57	120	130		140	150									
66	115	120	135	155	160									
89	110	120	130	130	100									
9	110		120		150	160								
39	105	100		125		130								
54	110	110	135		120	145								
62	100				140	145↑	160							
68	100	110			150		170							
70	100		120		140	125								
78	100	100		140		160								
93	100	100	115	125	125	120								
135	110			140		160								
15	110			150		150	150							
19	120	110	120	120	150	140	155							
44	105	120	125	135	140	155	160							
47	120	130	130	135	150	120	160							
64	100	100		130	125	140↑	150							
71	100	105	115		140	155	175							
75	100	100	110		130		135							
79	110	110	115	135	155	170	180							
86	100	110	115	110	125	130↑	120							
24	110	100	110	120	140	150	170↑	170						
29	110	120	140	150		195	180↑	160						
34	110			130		160	160↑	140						
65	105		110	140	150	180	180↑	140						
76	110		115	130	140	140	135	140						
84	125	125	130	135	120	130↑	135	120						
96	100		110	115	130	130↑								
97	115		125	140	150	160↑								
14	120	110			150↑	185↑	175	170	180					
22	120			120	135	150	145↑	130	185					
61	80	80	90	100	110	150↑		130	140					
32	115				125	180	150↑		170	170				
33	120				130	160	150↑	160	140	160				
35	110	100	115	130		120	100↑	105		115				
40	120			160		170	180↑	170	190↑	200	195			
73	100				120	130	130↑	125	130↑	125	125↑		160	170
Average elevation over preoperative level in mm. Hg.....		2	13	24	29	44	43	32	44	41	75	60	70	

Arrows indicate the animal was attached to the 'artificial kidney' on that day. Mean blood pressures in mm. Hg.

one ureter is ligated as shown in table 3 and fails to occur if one implants the ureter into the small intestine or vena cava as shown later. As seen in table 3, there is a rise in blood pressure following ligation of one ureter, the contralateral kidney and ureter

being left intact. Except in dog 88, which remained hypertensive during the 3 months it survived the operation, the blood pressure in the other animals returned to its normal preoperative level and remained so. In the case of dog 88, we are presumably dealing with the occasional animal in which unilateral nephrectomy, unilateral application of a figure-of-eight ligature, or other interference with renal

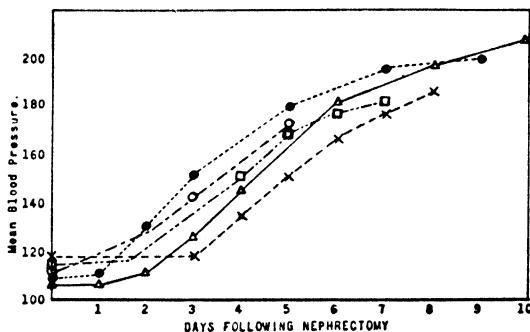


Fig. 1. MEAN BLOOD PRESSURE in mm. Hg of 5 dogs following bilateral nephrectomy. The animals were attached to the 'artificial kidney' for a period of 3 hours on the 5th day following nephrectomy.

TABLE 2. EFFECT OF LIGATING AND DIVIDING BOTH URETERS ON THE MEAN BLOOD PRESSURE OF THE DOG

DOG	PREOPERA-	DAYS FOLLOWING OPERATION									
		1	2	3	4	5	6	7	8	9	12
38	120	155	170								
51	105	120	145	150							
37	120		150	160	155						
49	120	120	140	145	180						
41	110		125	150	175	150↑					
27	110			110	230	145↑	120				
43	110	120	130	135	150	110↑	110				
45	115	110	140	170	150	130	110				
46	120	120	150	145	160	130↑	110	95			
23	120			160	135↑	130	160	200	190		
31	115				155	175	150↑	125	130	120↑	
100	120					120	110↑	100	100	120↑	120

Arrows indicate time when animal was attached to 'artificial kidney' for a period of 2 to 3 hours. Mean blood pressures in mm. Hg.

function as by ligation of the ureter suffices to induce permanent hypertension without removing the contralateral kidney as is usually necessary (1, 11).

Bilateral ureteral ligation not only stops the excretory activity of the kidney but also interferes with its blood supply because of pressure on the renal pelvis. There is congestion of the kidney, hydronephrosis and often perirenal hemorrhage. Nevertheless, certain activities of the kidney are apparently still preserved, for example, its glycogenic function (12) and presumably its capacity to maintain a normal blood pressure after subsidence of the acute rise which follows this operation.

In order to avoid the changes induced by ureteral ligation, the following observations in which the ureter was implanted into the gut or vena cava were performed.

*Effect of Implanting the Right Ureter in Small Intestine and Ablating Left Kidney.* As a control for the experiments cited in which both kidneys were removed, the right ureter was implanted into the duodenum or jejunum and several weeks later the left kidney was removed. The blood pressures of such animals as shown in Table 4 does not become elevated when the animals survive (by the aid of the artificial kidney), as in the case of dog 77, for as long as a month. The blood urea levels of such animals rise essentially to the same levels as those observed in bilaterally nephrectomized dogs as is evident from the data of table 5.

TABLE 3. EFFECT OF LIGATING AND DIVIDING ONE URETER, THE OTHER KIDNEY AND URETER REMAINING INTACT, ON THE BLOOD PRESSURE OF THE DOG

DOG	PREOPERATIVE LEVEL	DAYS FOLLOWING OPERATION									
		1	2	3	4	5	6	7	8	9	10
90	120	120	130	140	150	165	160	150	140	135	120
85	90	100	105	110	125	90	90	90	85	90	90
83	110	110	120	140	155	150	140	120	110	110	110
88	115	135	135	140	130	125	140	145	150	145	150
91	115	135	130	135	125	145	140	135	130	120	110
92	105	115	130	135	125	110	115	110	110	105	100

Mean blood pressure in mm. Hg.

TABLE 4. EFFECT OF IMPLANTING RIGHT URETER INTO THE SMALL INTESTINE AND REMOVING LEFT KIDNEY ON MEAN BLOOD PRESSURE OF THE DOG

DOG	PRIOR TO NEPHRECTOMY	DAYS FOLLOWING NEPHRECTOMY													
		1	2	3	4	5	6	7	8	9	10	11	12	15	30
121	110	110		100	110	100									
124	120				135	150									
126	110	110		110	120										
123	105	110	110	100	90	↑	70	80	80	↑	85	90	80		
74	105	100	105	110	110	90	100	↑	110	110	100	80	90	90	90
77	110	110	110		115	115	110	110	110	110	110	100	95	95	90

Arrows indicate application of artificial kidney. Mean blood pressures in mm. Hg.

Despite the rapid rise in non-protein-nitrogen of the blood which follows the implantation of a ureter into the duodenum and removal of the contralateral kidney, it may be objected that such preparations are not comparable to the bilaterally nephrectomized animal since some constituents other than the non-protein-nitrogenous bodies may not be reabsorbed from the intestine. Such an assumption is compatible with the observations of previous investigators who have noted that the degree of uremia and the period of survival of such animals is dependent upon the level at which the anastomosis is made. If made high in the duodenum, complete reabsorption of the urine occurs, there is a rapid increase in the non-protein-nitrogen level of the blood, and the animals survive for periods comparable to that following nephrectomy (13). On the other hand, if the transplant is made lower in the ileum,

there is no increase in the creatinine, creatine, uric acid or amino acid content of the blood and the animals survive for periods of 14 to 20 days (14).

*Effect of Implanting the Right Ureter in Vena Cava and Removing the Left Kidney.* In order to obviate the objections that the failure of the blood pressure to rise in dogs with a ureteral implant into the duodenum is due to the excretion by the bowel of certain catabolites, the series of experiments cited in table 6 were performed in which the ureter was implanted into the abdominal vena cava. Under these conditions no excretion is possible while relatively normal renal tissue remains in the organism.

TABLE 5. EFFECT OF IMPLANTING RIGHT URETER INTO THE SMALL INTESTINE AND REMOVING LEFT KIDNEY ON THE MEAN BLOOD PRESSURE AND BLOOD UREA LEVELS OF TWO DOGS

	DOG 74		DOG 77	
	Mean blood pressure mm. Hg	Blood urea level mg. %	Mean blood pressure mm. Hg	Blood urea level mg. %
Prior to removing left kidney.....	105	30	110	35
Days following nephrectomy				
1.....	100		110	
2.....	105	248	110	
3.....	110		110	320
4.....	110	415	115	
5.....	90	→ 475	115	512
6.....	100		110	317
7.....	110	→ 389	110	
8.....	110		110	428
9.....	100		110	→
10.....	80	→ 537	100	343
11.....	90		95	
12.....	90	520	100	471
13.....		→	110	514
15.....	90	600		→
16.....			115	610
21.....			95	→ 574
25.....			95	450
30.....			90	612

Arrows indicate days on which animals were attached to the artificial kidney.

As noted in table 6, the blood pressure under these conditions remains at its normal level.

#### DISCUSSION

In figure 2 are illustrated graphically the effects on the blood pressure of the various manipulations on the kidney carried out in the present investigation. The curves were constructed by averaging the differences between the blood pressure on each day following operation and the pre-operative levels. It is evident from figure 2 and the data of tables 1 to 6 that bilateral nephrectomy results in hypertensive blood pressure levels in the dog, and that in the presence of renal tissue in the body, as in the uretero-intestinal and ureterovenous anastomoses, despite the absence of

excretory function, the blood pressure remains at its normal level. When, however, the ureters are ligated there is an acute rise in blood pressure which returns to normal at a time when the blood pressure in the nephrectomized animal is still rising. The latter rise is generally accepted as being due to the liberation of a pressor agent (angiotonin or hypertensin) from ischaemic renal tissue. If only one ureter is ligated, this transient rise in blood pressure also appears and if the contralateral kidney is removed after the blood pressure returns to normal, no further elevation in blood pressure occurs.

TABLE 6. EFFECT OF IMPLANTING RIGHT URETER INTO VENA CAVA AND REMOVING LEFT KIDNEY ON THE MEAN BLOOD PRESSURE OF THE DOG

DOG	PRIOR TO NEPHRECTOMY	DAYS FOLLOWING NEPHRECTOMY											
		1	2	3	4	5	6	7	8	9	10	11	12
117	115		100	110	100								
112	130		120		130	135							
121	110	110		110	110	100							
118	110		120		140		120						
115	110			110	110		105		110				
113	115		120		130		130		135				
108	110	120		130	100	75	140	130	120				
104	110		100					135	130	125	110		

Arrows indicate application of artificial kidney. Mean blood pressures in mm. Hg.

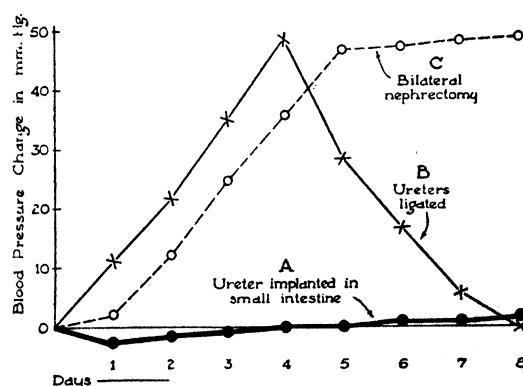


Fig. 2. AVERAGE CHANGES IN BLOOD PRESSURE of three series of 6 dogs; A, with the right ureter implanted into the small intestine and the left kidney removed (●—●); B, with both ureters ligated (x—x); and C, with both kidneys removed (○,—○). Note the constancy of the blood pressure in A, the transient rise with a return to normal levels in B and the continuous sustained rise in C. The ordinates indicate the changes in blood pressure over the preoperative levels; the abscissae, days following nephrectomy or ligation of the ureters.

Previous investigators have also noted the maintenance of a normal blood pressure level following the anastomoses of the ureters and the ileum (15) or the venous circulation (9, 15) and following bilateral occlusion of the renal veins (16). However, in these experiments the animals survived for only 3 days or less when all excretory function was abolished. Consequently, they are of little significance since one may justifiably attribute the observed results to the poor state of the animals or a failure of sufficient time to elapse for hypertension to manifest itself.

The rise in blood pressure to hypertensive levels of the nephrectomized dog obviously cannot have been due to the liberation of renin (hypertensin) or other pressor agents derived from the kidney since no renal tissue was present in the animal. Nor

can it be argued that the rise in pressure was due to any interference with normal hemodynamics by the use of the artificial kidney since the rise in pressure was evident even before the animal was subjected to this manipulation, and no rise occurred when uremic animals with renal tissue present were treated similarly.

The failure of previous investigators (17) to be impressed by the rise in blood pressure induced by nephrectomy must be attributed to the poor condition of their animals, as evidenced by the short period of their survival. As a matter of fact, perusal of the literature reveals instances of hypertensive levels in dogs following nephrectomy, as, for example, in the papers of Wintermitz, *et al.* (18), and Harrison, *et al.* (8) in whose series of nephrectomized animals several instances of definite hypertension occurred. However, since these rises occurred only occasionally, they were disregarded by these authors.

The present experiments offer direct evidence for the view that the kidneys exert an action other than their excretory function in the organism (19, 20). Not only is this demonstrated by the difference in the observed blood pressures in the presence and absence of renal tissue but also in the fate of the animals under the two conditions. The bilaterally nephrectomized animals, despite the removal of the excretory products by the 'artificial kidney', developed those pathological changes in the tissues which are associated with so-called malignant hypertension. These occasionally included hemorrhages in the eye-grounds and changes in the arterioles, skeletal muscles, myocardium and smooth muscle generally. Intussusception of the intestine which is common in dogs in which malignant hypertension is induced by the application of a clamp to one renal artery and removal of the opposite kidney occurred in over 10 per cent of our nephrectomized dogs surviving 8 days or more. A detailed pathological report of these studies and their implications will be reported elsewhere (5). They offer material evidence for determining the relative rôle played by the elevation in blood pressure, the accumulation of waste products and the presence or absence of renal tissue in inducing the pathologic changes observed in malignant hypertension.

Geer and Dragstedt (15) concluded from their observations on 4 dogs that the deviation of urine from one kidney into the blood stream produces no toxic symptoms. However, one of their 4 animals died on the 13th day while the others were killed on the 11th, 28, and 37th day. In our own series, 4 dogs in which the contralateral kidney was not removed died on the 7th, 12th, 13, and 30th days. It would appear therefore, that diversion of the urine into the blood stream is not entirely innocuous as appears also from our pathological studies of the tissues of these animals.

It may be objected that under the experimental conditions in which renal tissue is present without elevation in the blood pressure, the blood pressure fails to rise because of the detrimental effects of the experimental procedures. However, in cases of the uretero-intestinal anastomoses, the lesions present were much less pronounced than in those of bilaterally-nephrectomized dogs which in comparable periods of time manifest definite hypertension. In cases of the uretero-venous anastomoses, also, survival extended well beyond the time that elevations in blood pressure appear in the bilaterally nephrectomized animals. Our data also show that there is no close correlation between the elevation of the blood pressure and the severity of the lesions seen at autopsy.

## SUMMARY

The effect of the following procedures on the mean blood pressure of the dog was determined: 1) bilateral nephrectomy; 2) bilateral ligation of both ureters; 3) ligation of one ureter, the other remaining intact; 4) implantation of one ureter into the small bowel with removal of the contralateral kidney; and 5) implantation of one ureter into the abdominal vena cava with removal of the contralateral kidney. The period of survival of the animals deprived of renal excretory function was prolonged by maintaining them on an electrolyte-free diet and by application of an artificial kidney at intervals of 4 or 5 days.

The blood pressure of the nephrectomized animals gradually rose to hypertensive levels and at autopsy revealed the pathological findings of malignant hypertension. Ligation of the ureters resulted only in a temporary rise in blood pressure. If the contralateral kidney was removed following the return of the blood pressure to normal, no elevation in blood pressure occurred. Likewise, in animals with one ureter implanted in the small intestine or into the vena cava and the contralateral kidney removed, no hypertension resulted.

It is concluded that nephrectomy results in hypertension and that the presence of intact renal tissue is essential for the maintenance of the normotensive state. The present studies offer direct evidence for the view that the kidney normally, in addition to its excretory function, also exerts a function which is concerned in the maintenance of normal blood pressure levels. Hypertension of renal origin according to this view is not due to the liberation of a pressor agent, but results from a failure of this activity of the kidney.

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# EFFECTS OF EMOTIONAL DISTURBANCE ON WATER DIURESIS AND RENAL BLOOD FLOW IN THE RABBIT<sup>1</sup>

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**I**N THE dog and man under normal conditions, variations in urine flow are to a great extent independent of the glomerular filtration rate, water diuresis being effected primarily by decreased tubular reabsorption (1). In rabbits, however, Kaplan and Smith (2) reported that filtration rate and urine flow varied in a parallel manner, water diuresis being accompanied by an increase in the filtration rate. These observations, which have been confirmed by Dicker and Heller (3) and Forster and Maes (4), place the rabbit in an anomalous position among the mammals so far investigated. Our reinvestigation of this problem was occasioned by the extreme difficulty which we encountered in some experiments in producing water diuresis in certain rabbits, and further study has led us to the conclusions that the apparent relationship between filtration rate and urine flow in the rabbit is physiologically fortuitous, in that when present it is attributable to a reversible reduction in filtration rate, associated with renal ischemia reflexly induced by the manipulation of excitable animals; as the renal and glomerular circulation recover, the urine flow increases *pari passu* with the filtration rate. In the absence of renal ischemia, variations in urine flow appear to be mediated independently of the filtration rate, as in man and the dog.

## METHODS

Simple water diuresis experiments were performed on 42 unanesthetized adult male rabbits weighing from 2300 to 4400 gm. The animals were maintained on a diet of dry oats and dried greens and had free access to water. Depending upon the conditions of the experiment, they were either kept in a closed box or tied to an animal board throughout the period of observation. For the induction of water diuresis they were given 50 cc. distilled water per kg. body weight by stomach tube. Urine was obtained through an indwelling no. 16 French Foley catheter with a 5-cc. bag in the bladder inflated with water to insure against expulsion of the catheter. After each collection period the bladder was washed with 5 cc. of distilled water and 10 cc. of air. The efficiency of this method of bladder emptying was confirmed at numerous post-mortem examinations which failed to reveal any residual bladder urine.

The filtration rate was measured by the exogenous creatinine clearance (2), and the effective renal plasma flow by PAH (p-aminohippuric acid) clearance (5). Priming and maintenance doses of these substances were calculated to maintain plasma levels of 30 mg. per cent for creatinine and 2 mg. per cent for PAH. The sustaining dose was given as a constant intravenous infusion in isotonic saline into the marginal vein of the ear at a rate of 0.3 cc. per minute by means of a mercury gravity

Received for publication January 31, 1949.

<sup>1</sup> This work was aided by a grant from the Rockefeller Foundation.

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drip pump. The priming dose was injected directly into the rubber tubing feeding the sustaining infusion. One hour was allowed to elapse from the time of the priming dose before starting the first urine-collection period in order to insure equilibrium levels of the test substances throughout the body fluids. Urine-collection periods varied from 10 to 60 minutes in length, depending upon the urine flow. Blood samples were obtained by means of cardiac puncture. Blood pressure measurements were recorded by the method of Grant and Rothschild (6). Creatinine was determined by the standard Folin (7) method and PAH by the technique of Smith *et al.* (5). Emotional disturbances were created by faradic shocks applied to the bare skin of the thorax; by loud, banging noises; by pinching; and, in hyperexcitable animals, by merely keeping them tied to an animal board in the supine position. When freedom from emotional stress was desired the rabbit was placed upright in an enclosed box, allowing free egress of the head, and disturbing sounds were avoided.

### RESULTS

*Effect of Emotional Stress on Water Diuresis.* Water diuresis was induced in a group of hyper-excitable rabbits when secured to an animal board in the supine position, and compared with the diuresis obtained in the undisturbed condition. This stimulus was sufficient to produce obvious tenseness and hyper-irritability for 5 hours, with the exception of *rabbit 10*, table 1, which was conspicuous by its docility. The results of 13 water-diuresis experiments on the animal board and in the apparently non-disturbing environment of the box are contrasted in table 1 and figure 1. The difference in diuretic response is very marked. The average urine output in a 5-hour period of the 7 animals on the board was 24.8 per cent of the total water ingested, as compared to 87.1 per cent for the animals in the box. The good diuretic response obtained with *rabbit 10* on the board seemed to be related to his apathy or docility.

The supine position alone does not seem to affect water diuresis, since the same degree of inhibition of diuresis was obtained when the animals were tied to the board in the prone position. Forster and Maes (4) also noted that variations of the horizontal position resulted in little change in the renal function of rabbits.

*Effect of Emotional Stress and Painful Stimuli upon Renal Blood Flow.* Water diuresis was induced by placing the rabbits in the box after water administration, and at the height of the diuresis the animals were disturbed by unpleasant stimuli. The results of 15 such experiments are recorded in table 2, and the course of a typical experiment is shown in figure 2. In all cases the disturbing stimuli were immediately followed by a marked decrease in urine flow, effective renal plasma flow and filtration rate, and a more gradual increase in the creatinine U/P ratio, representing increased tubular reabsorption of water. There was no consistent change in the filtration fraction; in 8 instances it was elevated, in 4 it was decreased, and in 3 there was no significant change. The blood pressure, if it changed at all, increased by 5 to 20 mm. Hg following the stimulus. Oliguria was frequently very severe, lasting from 30 to 120 minutes. The experiment was terminated in 6 cases by death of the animal in convulsions, apparently because of water intoxication resulting from prolonged oliguria in the face of excessive hydration coupled with prolonged intravenous infusion at the rate of 0.3 cc. per minute. These convulsive deaths were similar to those observed by Rowntree (8) in his study of water intoxication. Post-mortem examination revealed only distention of the venae cavae, and in only one instance was pulmonary edema present. Although a few of the animals tested were relatively

resistant to excitation, oliguria could always be provoked by increasing the strength of the stimulus.

From these data it appears that the oliguria associated with painful stimuli and emotional stress in the rabbit is caused primarily by a reduction in renal blood flow and filtration rate and to only a minor degree by increased tubular reabsorption of water.

TABLE I. EFFECT OF EMOTIONAL STRESS UPON WATER DIURESIS

RABBIT NO.	PER CENT OF INGESTED FLUID EXCRETED IN 5 HOURS On Board	PER CENT OF INGESTED FLUID EXCRETED IN 5 HOURS In Box
2	16.6	102
3	21.4	106
6	7.00	
7	4.80	
8		70.0
9	11.5	63.0
10	83.0	
11	29.6	102
14		79.5
Average	24.8	87.1

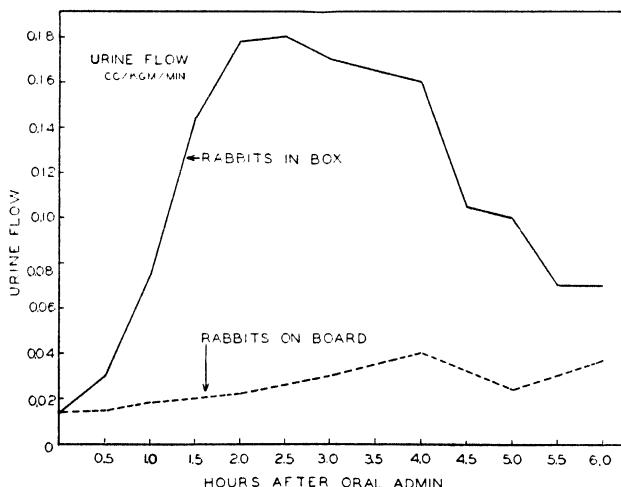


Fig. 1. COMPARISON OF AVERAGE WATER DIURESESIS CURVES obtained with excited and quiescent rabbits, revealing the inhibition of water diuresis in the disturbed animals.

*Relationship between Glomerular Filtration Rate and Urine Flow in the Unexcited Rabbit.* Animals which appeared to us to be least disturbed by handling were chosen for this series of experiments, and the environment was maintained free of disturbing stimuli. The results of 8 such experiments are tabulated in table 3 and figure 3. The data show that with such selected animals and under suitable conditions it is possible for the urine flow to vary as much as 16-fold during water diuresis

without significant changes in the filtration rate. The normal variation in urine flow in the unexcited rabbit appears to be a function of tubular reabsorption of water, as in the other common mammals.

*Factors Controlling Effect of Emotional Stress and Painful Stimuli on Renal Blood Flow.* Three possible mechanisms were considered: 1) general circulatory effects; 2) stimulation of renal vasomotor apparatus by neural transmission; 3) stimulation of renal vasomotor apparatus by humoral factors.

TABLE 2. EFFECT OF EMOTIONAL STRESS AND PAINFUL STIMULI ON RENAL CIRCULATION

RAB- BIT NO.	QUIET PERIOD						PERIOD OF EMOTIONAL STRESS						RATIO OF FUNCTIONS BEFORE AND AFTER					
	Urine Flow Be- fore Stim-	Creat. no. plus	PAH Clear.	U/P Ratio	Fil- tra- tion	Type of Stim- ulus	Urine Flow Follow- ing Stim- ulus	Creat. no. plus	PAH Clear.	U/P Ratio	Fil- tra- tion	Urine Flow Clear	Creat. no. plus	PAH Clear.	U/P Ratio	Fil- tra- tion		
	cc/ min.	cc/ min.	cc/ min.				cc/ min.	cc/ min.	cc/ min.				cc/ min.	cc/ min.	cc/ min.			
on board																		
10 <sup>1</sup>	1.40	7.75	45.5	5.55	0.170	supine faradic	0.252	2.34	12.1	9.3	0.193	5.55	3.31	3.77	0.598	0.881		
3 <sup>1</sup>	0.706	0.52	40.1	13.6	0.237	shock on board	0.067	2.21	10.2	33.0	0.217	10.5	4.31	3.93	0.412	1.09		
13 <sup>1</sup>	0.930	7.50	27.4	8.00	0.274	prone heart	0.037	0.637	2.53	17.3	0.252	25.4	11.8	10.8	0.462	1.09		
II <sup>1</sup>	0.146	7.88	89.0	54.0	0.080	puncture heart	0.064	2.04	21.5	33.4	0.095	2.28	3.86	4.14	1.62	0.932		
I7 <sup>1</sup>	0.900	15.2	199.0	21.7	0.078	puncture escape	0.28	9.25	12.2	33.0	0.076	3.21	1.64	1.63	0.658	1.03		
25	1.50	14.4	85.7	9.60	0.168	reaction faradic	0.59	7.93	21.5	13.3	0.368	2.54	1.82	3.98	0.794	0.457		
25	0.590	7.93	21.5	13.3	0.368	shock faradic	0.063	2.96	6.06	47.2	0.488	9.37	2.68	3.55	0.284	0.754		
22	0.606	11.6	38.1	19.1	0.305	shock heart	0.300	8.61	14.7	28.7	0.585	2.02	1.35	2.59	0.666	0.522		
22	0.426	11.7	29.9	27.4	0.293	puncture tube	0.076	3.09	11.05	40.6	0.280	5.61	3.79	3.62	0.675	1.05		
155	0.222	11.1	66.4	52.0	0.168	feeding tube	0.109	6.70	29.2	61.5	0.230	2.04	1.66	2.28	0.846	0.731		
158	0.1320	11.9	82.0	90.0	0.145	feeding on board	0.059	7.16	39.4	121.3	0.181	2.24	1.66	2.08	0.741	0.802		
164	0.191	8.80	27.4	46.0	0.321	supine tube	0.037	3.08	10.4	83.3	0.298	5.16	2.86	2.64	0.552	1.08		
157 <sup>1</sup>	0.299	6.30	24.1	21.0	0.261	feeding tube	0.145	3.59	18.4	24.8	0.195	2.06	1.75	1.31	0.844	1.34		
150	0.132	11.9	82.0	90.0	0.145	feeding heart	0.059	7.16	39.4	121.1	0.181	2.24	1.66	2.08	0.744	0.800		
150	0.934	7.78	43.8	8.33	0.178	puncture	0.071	4.56	20.3	6.4	0.225	13.1	1.71	2.16	0.130	0.792		

<sup>1</sup> These animals died with convulsions, apparently due to water intoxication.

- 1) Since the blood pressure of all the animals tested remained either constant or revealed a moderate rise, the depressed renal blood flow could not be attributed to decreased blood pressure in any instance. 2) In order to investigate the effect of neural impulses transmitted via renal nerves, we repeated the excitation experiments in 5 rabbits with denervated kidneys. Both kidneys were denervated by stripping all visible strands of tissue from the hilar vessels and ureters, followed by local application of 5 per cent phenol for 2 minutes. The experiments were performed from 10 to 14 days post-operatively. The results of these experiments duplicate those in

unoperated animals: painful stimuli and emotional excitement caused a marked reduction in diuresis, filtration rate and plasma flow. Our results indicate that the renal nerves are not necessary for the production of renal ischemia.

3) Several experiments were designed to investigate the influence of administered antidiuretic hormone and adrenalin in the production of oliguria. At the height of water diuresis under quiescent conditions, 2 millionunits of pitressin were administered intravenously to 4 rabbits weighing about 2500 gm. This dose was sufficient to produce antidiuresis in 3 of the 4 animals, with only slight and transitory decreases in the renal plasma flow and filtration rate in 2 of the 3. The inhibition of diuresis

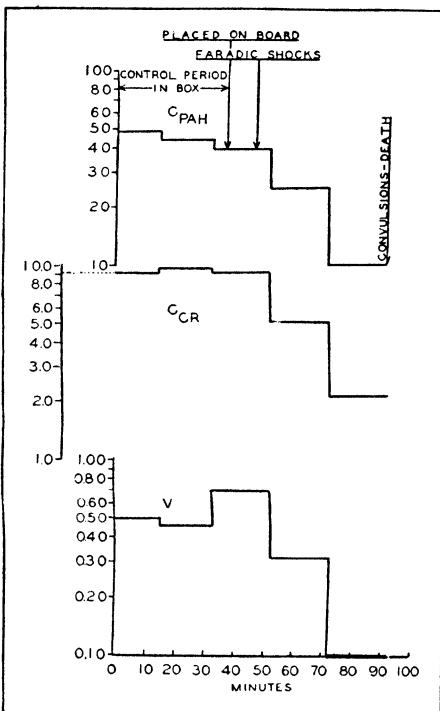


Fig. 2. COURSE OF A TYPICAL EXPERIMENT in which faradic shocks were applied at the peak of water diuresis, marked parallel depression of renal blood flow, glomerular filtration and urine flow results.

was due primarily to increased tubular reabsorption of water. The respective changes in the creatinine U/P ratios were 26 to 73, 28.5 to 64.8 and 14.8 to 45.0. In contrast, 400  $\gamma$  of epinephrine caused a marked reduction in renal plasma flow and filtration rate and marked oliguria, with little effect upon the creatinine U/P ratio. In one rabbit the urine flow decreased from 0.162 to 0.065 cc/min., the renal plasma flow from 33.9 to 5.92 cc/min., and the filtration rate from 5.20 to 1.71 cc/min., with the creatinine U/P ratio changing from 32.1 to 26.4. Epinephrine in doses of from 50 to 120  $\gamma$  failed to produce consistent effects. It appears that the oliguric state stimulates the renal effects of relatively large doses of epinephrine. Dibenamine (9) was administered to 7 animals at the height of oliguria to determine whether this

TABLE 3. VARYING URINE FLOW WITH RELATIVELY CONSTANT GLOMERULAR FILTRATION IN UNDISTURBED RABBITS

2	3			22			26			38			47			48			55			
	Urine Flow	Creat. U/P	Creat. Clear.																			
0.033 2.55	76.5	0.397	3.72	12.1	0.060	3.34	56.0	0.041	3.54	85.3	0.336	3.67	11.1	0.182	2.63	14.8	0.008	6.70	68.5	0.074	3.71	52.0
0.031 2.42	77.0	0.053	3.12	58.8	0.065	3.51	54.5	0.029	2.34	80.0	0.320	3.42	10.7	0.226	2.59	11.7	0.070	6.41	21.7	0.073	3.24	44.3
0.025 2.39	95.0	0.054	3.66	67.7	0.044	2.90	66.7	0.220	3.81	17.2	0.121	2.06	24.3	0.199	2.97	15.0	0.379	4.38	11.6	0.097	3.71	38.3
0.049 2.89	59.0	0.018	2.69	15.0	0.208	5.04	24.3	0.267	3.11	11.7	0.054	3.07	57.5	0.191	2.84	14.9	0.180	3.90	33.0	0.326	3.71	11.4
0.069 2.45	35.5	0.003	3.82	68.7	0.188	3.58	18.5	0.338	3.34	9.85	0.055	2.95	53.3	0.082	2.27	27.8	0.098	7.54	77.0	0.267	3.17	11.9
0.201 2.06	13.3	0.042	3.61	86.0	0.207	3.95	19.1	0.390	3.30	10.7	0.099	4.38	44.1	0.000	2.68	45.0	0.126	5.27	41.5	0.209	3.14	15.1
0.297 2.75	9.25	0.191	3.53	18.5	0.102	2.94	28.7	0.165	3.12	18.8	0.038	3.52	91.2	0.040	2.45	60.7	0.174	7.08	40.8	0.128	3.44	26.5
0.217 2.46	11.4	0.176	3.65	20.8	0.149	3.73	25.5	0.091	2.96	32.9	0.031	2.95	95.4	0.230	6.54	28.5	0.236	3.51	14.8			
0.230 2.78	12.9	0.270	3.63	13.6	0.270	4.98	10.6	0.076	3.81	50.0	0.054	2.84	53.0									

All urine flow and creatinine clearance figures are expressed as cc/kg/min. of body weight.

adrenolytic agent had any effect upon the mechanism. The drug was given intravenously in doses of 5 mg/kg. of body weight. In 3 rabbits with intact renal nerves this drug had no significant effect. In 3 of the 4 animals with denervated kidneys there was a definite increase in the renal plasma flow and filtration rate immediately following its administration, with no change in the 4th. The effect of dibenamine upon the action of large doses of adrenaline was not determined in either group of animals.

#### DISCUSSION

O'Connor and Verney (10) have shown that painful stimuli in dogs produce two types of inhibition on water diuresis: a rapid, short-lasting inhibition which is pre-

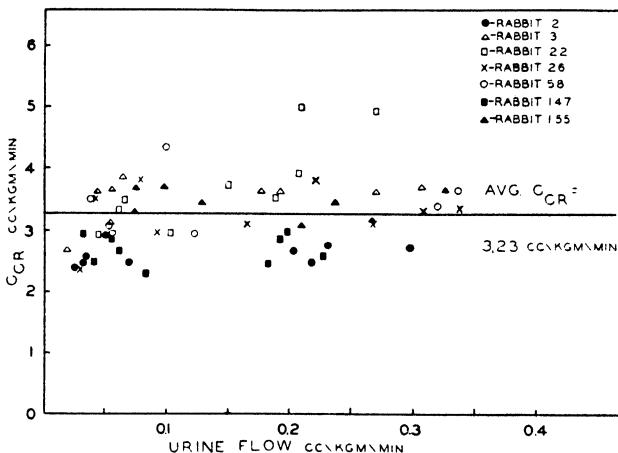


Fig. 3. RELATIONSHIP BETWEEN CREATININE CLEARANCE AND URINE FLOW in undisturbed rabbits. A 16-fold increase in urine flow during water diuresis is possible without significant variation in creatinine clearance. Rabbit 148 (table 3) is not included in this figure because of an unexplained high creatinine clearance averaging 5.98 cc/kg/min.

vented by section of the splanchnic nerves or by denervation of the kidneys and adrenals; and a slow, prolonged inhibition which is abolished by hypophysectomy or section of the supraopticohypophysial tracts. Haterius (11) has shown that antidiuresis is produced in anesthetized rabbits by painful stimulation of the lumbar region and he attributed the phenomenon to increased secretion of the antidiuretic hormone, since the response was abolished by destruction of the pituitary stalk, but he states that, with excessive struggling, antidiuresis may occur in the absence of the pituitary. He also cites a personal communication from Ingraham to the effect that inhibition of diuresis may occur in cats with diabetes insipidus following unpleasant handling, such as repeated catheterization or gavage. Lippman (12) demonstrated that the painful 'tailcutting' method of obtaining blood in rats reduced PAH clearances by 14 per cent and creatinine clearances by 37 per cent below those values obtained with the painless 'undisturbed' method. Smith (13) and Wolf (14) have

demonstrated reduction in renal plasma flow in man after psychological and physical trauma.

It is clear that antidiuresis may involve either an increased secretion of the anti-diuretic hormone or renal ischemia, or both. In contrast to O'Connor and Verney's observations in dogs, our data on rabbits indicate that antidiuresis occasioned by disagreeable stimuli in this species is to be attributed primarily to renal vasomotor changes which cause a marked and prolonged reduction in renal plasma flow and filtration. The absence of any marked increase in the reabsorption of water, as indicated by the creatinine U/P ratio, argues against a significant contribution from the antidiuretic hormone. Apparently marked sympathomimetic activity is easily elicited in the rabbit in contradistinction to the dog.

The locus of vasoconstriction in the rabbit has not been determined, but application of Gomez' (16) equations to our data on renal plasma flow and filtration rate in animals where the blood pressure was measured indicates that both the effective efferent and afferent resistances increase about equally.

Under the conditions of our experiments, one might expect that the renal blood flow might be diverted through the juxtamedullary glomeruli, as described by Trueta and his colleagues (15), in which case one would anticipate a marked reduction in the renal extraction ratio of PAH. Several attempts to measure the extraction ratio by the collection of renal venous blood were defeated by almost complete renal ischemia. However, during oliguria in 4 of our rabbits we obtained good and uniform x-ray visualization of the renal vascular tree following the administration intravenously of a large dose of diodrast. If some of the glomeruli were excluded from the circulation they were uniformly dispersed in areas which still received good circulation through adjacent glomeruli.<sup>4</sup>

Our data show that in a non-excited rabbit a considerable variation in urine flow (0.02 to 0.32 cc/kg/min.) accompanies water diuresis without any related change in filtration rate. Thus the uncomplicated diuretic response appears to be a purely tubular phenomenon, as in the dog and man.

We believe that the parallel variation in urine flow and filtration rate reported by other authors (2-4) is attributable to a reduction in renal blood flow occasioned by the experimental procedures. The majority of observations made by Dicker and Heller (3) and Forster and Maes (4) were made during rising diuresis which corresponds to the recovery phase from the oliguria induced by the administration of water and other procedures necessary to initiate the experiments. Observations made at various stages of this recovery process yield a fortuitous correlation.

#### SUMMARY

Emotional disturbance and painful stimuli in the rabbit cause antidiuresis by decreasing the renal blood flow (PAH clearance) and filtration rate (creatinine clearance). Increased tubular reabsorption of water (as judged by the creatinine U/P ratio) is of minor importance in this oliguria. The strength of the stimulus needed

<sup>4</sup> We are indebted to Dr. Charles Gottlieb, Chairman of the Department of Radiology, New York University College of Medicine, for making the facilities of his department available to us.

to produce emotional oliguria varies with the excitability of individual animals, but the mechanism may invariably be provoked by sufficiently strong stimuli. During emotional oliguria, water-intoxication convulsions and death are easily provoked.

When emotional disturbance is avoided, the urine flow may vary 15-fold with a constant filtration rate. Pitressin in physiological doses causes a marked increase in the tubular reabsorption of water with little effect upon renal blood flow. Adrenalin in large doses causes renal ischemia similar to that observed during emotional oliguria. Emotional oliguria may be induced with equal facility in rabbits with enervated and denervated kidneys.

#### ADDENDUM

Since the completion of this work, Wills and Main (17), working with anesthetized rabbits found constant filtration rates with varying urine flows, thereby agreeing with our results as obtained with unanesthetized and undisturbed rabbits.

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# RESPONSE OF RENAL BLOOD FLOW AND CLEARANCE TO GRADED PARTIAL OBSTRUCTION OF THE RENAL VEIN<sup>1</sup>

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**I**NVESTIGATION of the rôle of the kidney in the edema of congestive heart failure has suggested a sodium retaining mechanism whereby both glomerular filtration rate and renal blood flow are reduced, the latter more so than the former, while tubular reabsorption of filtered sodium is unimpaired (1, 2). Since glomerular filtration rate is not reduced as much as concurrent renal plasma flow the filtration fraction is elevated. Opinion differs as to whether the increased filtration fraction is due to efferent arteriolar vasoconstriction presumably on a humoral basis (3, 4) or due to high glomerular capillary pressure resulting from the elevated venous pressure which usually accompanies congestive heart failure (5). Merrill dismisses the venous pressure as being unimportant in the renal changes. Bradley and his associates (6, 6a) found, however, that when renal venous pressure in human subjects was elevated from 5.8 mm. Hg to 18.3 mm. Hg, effective renal plasma flow and glomerular filtration rate were reduced on the average by 24.4 and 27.5 per cent respectively. They found no significant change in filtration fraction. Bradley obtained elevation of renal vein pressure by inflation of a pneumatic abdominal girdle to 80 to 100 mm. Hg. This transmitted a pressure of 20 mm. Hg to the abdomen.

Merrill found no significant correlation between venous pressure levels and inulin and hippurate clearances in different patients. Yet in view of the findings of Bradley it would seem likely that alterations in renal vein pressure might have a definite effect on renal clearance when other factors which might influence renal function are kept constant. However, since the abdominal cuff technique might introduce other modifying factors on the kidney function, such as vasomotor influences of psychogenic origin and compression of the ureters and renal pelvis on rate of urine flow, it seemed desirable to investigate the influence of renal vein obstruction on renal function by a technique specific for that factor alone. This was accomplished in dogs by exposure of the renal vein and gradual occlusion by a tourniquet. Renal vein pressure was recorded from a point between the obstruction and the kidney. Effects on either direct blood flow or clearances of creatinine and p-amino-hippurate (PAH) were studied.

## METHODS

Dogs were anesthetized with 30 mg/kg. pentobarbital sodium administered intravenously. In one group the influence of venous obstruction on direct renal blood flow was examined with an

Received for publication January 14, 1949.

<sup>1</sup> Supported by a grant from the Division of Research Grants and Fellowships, U. S. Public Health Service.

optically recording bubble flow meter by the arterial inflow technique in adequately heparinized animals. This method is described in detail elsewhere (7). In this group, exposure of the left kidney was made by a dorsal retroperitoneal approach. Renal arterial pressure was measured from a point just proximal to the renal arterial cannula. Renal vein pressure was obtained by aid of a specially designed metal sound which reached from the right jugular vein into the left renal vein. Zero levels for venous pressure were set at the level of the inferior vena cava, and for arterial pressure at the level of the renal artery. Pressures were recorded by modified Gregg optical manometers of suitable sensitivity and frequency. To produce elevation in renal vein pressure a tourniquet was passed around the renal vein medial to the end of the inlying renal vein sound. This tourniquet was passed through a rigidly supported brass tube to the exterior to permit gradual uniform constriction of the vein.

In a second group, renal clearances were performed with suitable blood levels of creatinine and PAH maintained by intravenous infusion or subcutaneous injection. Mannitol was included to act as an osmotic diuretic. In these animals a ventral approach to the renal vein was used. The method of measuring renal vein pressure and producing graded venous pressure elevation was the same as above, except that a water manometer was used instead of optical registration for recording pressures. In this group femoral arterial pressure as taken by a mercury manometer was used to calculate the A-V pressure differences across the renal vascular circuit. Urine was collected by direct cannulation of the left ureter. This minimized dead space and emptying errors. The alkaline picrate method (8) was used for creatinine analysis of urine and sodium tungstate plasma filtrates. Analysis for PAH was by the method of Smith (9) on CdSO<sub>4</sub> plasma filtrates and urine. All analyses were made in duplicate. Clearances were calculated by obtaining approximate midpoint plasma values by interpolation to the slope established by samples taken before and after each pair of urine collection periods of ten minutes duration. Appropriate correction was made for emptying delay.

The general plan of the experiment was to raise renal venous pressure in three stages following a suitable control period. In the experiments concerned with direct blood flow readings were taken every two minutes for a total period of 10 to 12 minutes at each stage, with brief periods intervening for tourniquet adjustment and stabilization of flow. Following the highest level of venous pressure elevation the tourniquet was released and usually two recovery periods were obtained. In the clearance experiments, each phase consisted of two consecutive urine collection periods preceded by adequate discard periods.

## RESULTS

*Effect of Graded Venous Obstruction on Direct Renal Blood Flow.* Five control experiments were performed to establish possible effects of experimental procedures without venous obstruction on blood flow as measured by the bubble flow meter connected between the carotid and renal artery. In all cases blood flow decreased somewhat during periods of time equivalent to experiments in which venous pressure elevation was produced. From this evidence it seemed necessary to compare the renal blood flow during the periods of elevated venous pressure with the expected mean trend, rather than to the initial control value. The basis for this is the fact that the decline in flow observed in the control series is a linear function which can be predicted by a line connecting the initial and final periods. This is illustrated in the representative experiment in figure 1-A. In the five control experiments 25 intermediate periods averaged 0.98 of the predicted trend. The effect of graded venous obstruction was accordingly measured by deviation of flow from the line connecting the control average with the recovery average, and expressing the experimental change as a ratio to this predicted mean trend. In the succeeding discussion this ratio will be referred to as the *experimental/control ratio*, it being understood that the designation 'control' here does not refer to the initial control value, but rather to the adjusted value based on the expected mean trend.

A representative experiment showing the effects on renal flow of graded elevation of renal venous pressure is shown in figure 1B. For three successive stages of venous pressure elevation the experimental/control ratio is 0.91, 0.93, and 0.81. Because all seven experiments differed somewhat in control rate of flow (range, 69 to 134 cc/min/kidney; average, 119 cc/min.) and in control renal arterial pressure (range, 72-121 mm. Hg; average, 95 mm. Hg), the changes in flow are combined graphically in figure 2, where percentile deviation from control flow is related to the renal venous pressure. It is seen that effects on flow are significant in 3 experiments

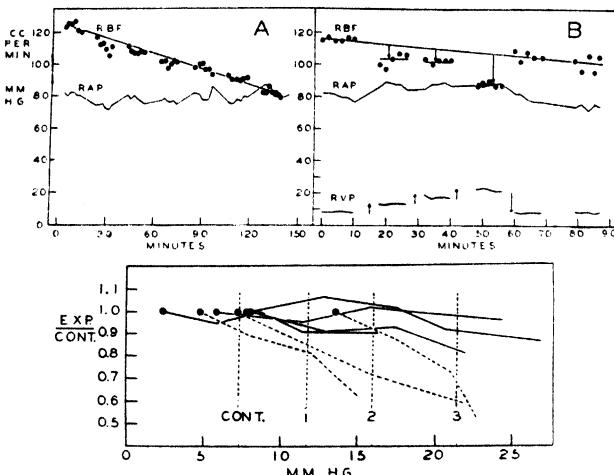


Fig. 1. (upper) (A) THE CHANGE IN DIRECT RENAL BLOOD FLOW (RBF) in cc/min/kidney in a typical experimental preparation. This is considered as the 'control' trend, for the gradual decrease in flow noted is due to nonspecific factors other than renal venous pressure elevation. RAP: = renal arterial pressure.

(B) THE EFFECT OF ELEVATION of renal venous pressure (RVP) on direct blood flow. The upper arrows show the degree of reduction of RBF from the expected trend at each stage of RVP elevation.

Fig. 2 (lower). SUMMARY OF THE EFFECT OF ELEVATION OF RVP (in mm. Hg on the abscissa) on direct blood flow expressed in a ratio to the control value. Solid circles are the control renal venous pressures. Vertical lines represent the average renal venous pressures of successively the control, first, second, and third stages of venous elevation. The sloping dashed lines show experiments in which it is believed that other factors (neurogenic or humoral) are superimposed on the effect of venous obstruction, accounting for the more marked effect (see text).

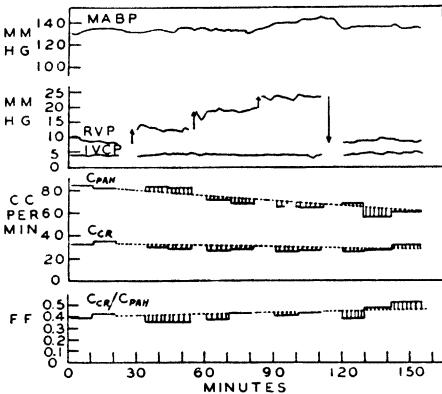
(dashed lines in figure 2) with experimental/control ratios of 0.53, 0.59, and 0.63, but less significant in the other 4 (0.81 to 0.96) at the highest venous pressures. The average decrease in flow for all was 18 per cent in a range of venous pressures from the control average of 7.3 mm. Hg to the average of the third stage of venous elevation, 21.3 mm. Hg. The average decline in pressure gradient across the renal vascular circuit (arterial pressure minus venous pressure) was from 88 to 79 mm. Hg, a 10 per cent decrease.

Calculation of renal vascular resistance from the ratio  $\frac{P(A-V)}{F}$  showed increases

of 28, 60, and 80 per cent respectively in the above 3 experiments which showed significant reductions in flow. The other 4 showed no significant changes (+13 to -10 %), indicating that the decline in flow in these experiments was simply the result of decreased perfusion pressure unaccompanied by change in renal vascular resistance in the range observed. Inasmuch as this is true in 6 experiments to be described subsequently in which renal flow was estimated from the PAH clearance, it can be concluded that the typical effect of elevation of renal venous pressure on the renal circulation is a decline in flow directly proportional to the decrease in effective pressure gradient across the renal circuit. Apparently, humoral or vasomotor factors were superimposed upon the effects of venous obstruction in the above 3 exceptions.

*Effect of Graded Venous Obstruction on Renal Clearances.* Although study of the renal blood flow by direct methods has advantages in objectivity it was thought that examination of renal clearances under similar experimental conditions would

Fig. 3. EFFECT OF ELEVATION OF RVP ON clearance of PAH and creatinine, and on the filtration fraction (FF). This experiment is one in which the changes in the clearances from the expected trend are minimal. Clearance values are for one kidney. Mean arterial pressure (MABP) was taken at the femoral artery. IVCP: inferior vena cava pressure.



throw light on the rôle of elevated venous pressure on the filtration fraction. This was done to test the postulate that increased venous pressure might increase glomerular capillary pressure and hence the filtration fraction. By using the clearance of PAH to estimate renal plasma flow further information was supplied on the effect of venous pressure elevation on blood flow.

As was done with the data on direct blood flow, it appeared wise to consider the possible effects of experimental procedure other than renal vein obstruction on renal clearances. Data are available from 10 animals observed in connection with another study which were subjected to similar anesthetic and surgical procedures. The average decline in PAH clearance was 15 per cent (range, -1 to -40) and 10 per cent for creatinine (range, 0 to -30 %) during an average time interval of 198 minutes. Intermediate periods (PAH) averaged 93 per cent of the expected trend.

Comparison of the recovery periods following partial venous obstruction with the control averages in the present series showed a decrease of 27 per cent (-6 to -43) for PAH and 20 per cent (+2 to -39) for creatinine. Although these changes average greater than the above control data there is considerable overlap, and the

view is favored that the changes are due to other factors than the specific effect of renal vein obstruction. For this reason, experimental changes resulting from elevated venous pressure will be compared to the expected trend set by the control and recovery periods, as was done with the direct blood flow experiments.

Two representative experiments illustrating the extremes of results are graphically presented. In figure 3 the trend of PAH and creatinine clearances appears to be only slightly modified by renal venous pressure elevation, while in figure 4 the effect of increased venous pressure is significant. The difference in effects is readily explained on the basis of the change in effective pressure gradient (A-V). In figure 3, due to a fortuitous rise in mean arterial pressure, the net decrease in pressure was only 6.5 per cent during the third stage of venous pressure elevation. This corresponds with a decrease in PAH clearance of only 4.0 per cent. On the other hand, in figure 4 arterial pressure declined during the course of the experiment, and the

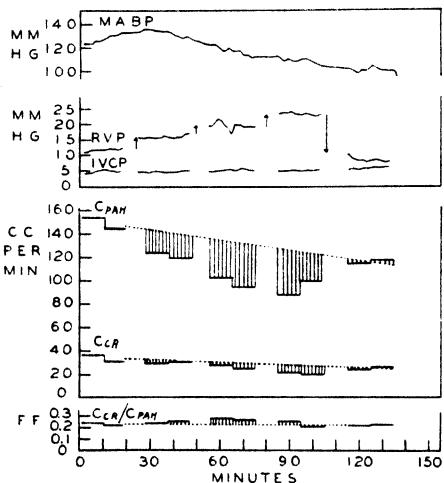


Fig. 4. THIS EXPERIMENT is one in which the effect of elevated RVP on the expected trend of  $C_{PAH}$  and  $C_{CR}$  was greatest.

A-V pressure gradient decreased by 25 per cent during the third stage of venous elevation; during this phase the PAH clearance was 26 per cent below the expected trend. Thus it is clear that when changes in effective plasma flow occur they are directly related to changes in renal perfusion pressure.

In figure 5 the effects on PAH clearance are graphically combined for all 6 experiments in terms of percentile deviation from the control. As venous pressure was elevated from the control average of 7.7 mm. Hg to 23.5 mm. Hg, the clearance of PAH decreased by an average of 12 per cent from the expected trend: range (-4 to -26%). The average calculated A-V pressure change across the renal vascular circuit decreased from 125 to 108.5 mm. Hg at the highest venous pressures, a 13 per cent decrease. This signified no change in renal vascular resistance, since flow declined proportionally to the average decrease in effective pressure gradient in all cases.

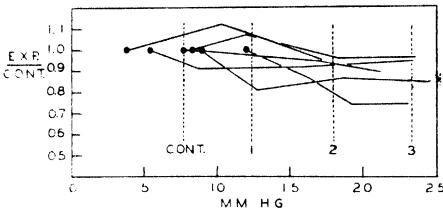
The changes in the clearance of creatinine paralleled the changes in the PAH clearance in individual experiments and the average decrease at the highest venous

pressure was also 12 per cent. Thus it is obvious that there was no significant change in filtration fraction resulting from venous pressure elevation in the range observed.

#### DISCUSSION

The height of venous pressure elevation in most of these experiments (ca. 29-36 cm. water) was comparable to the degree of venous pressure elevation seen in severe congestive heart failure. Yet with this degree of elevation the reduction in renal blood flow was only 18 per cent in a group of animals in which flow was measured directly, and in another group clearances of PAH and creatinine were reduced only by 12 per cent.<sup>2</sup> This would appear to dismiss elevated venous pressure as the factor which causes the marked reduction in effective plasma flow (PAH clearance) and glomerular filtration rate in clinical congestive heart failure. It is possible that higher degrees of venous pressure elevation than those investigated would have more significant effects on renal blood flow and glomerular filtration rate, but by extrapolation of the present trend it would appear that venous pressure elevation of at least three times that produced in the present experiments would be required to bring glomer-

Fig. 5. SUMMARY OF THE EFFECTS OF ELEVATED RVP ON the clearance of PAH expressed as a ratio to the control value in successive stages of elevation (vertical lines represent average renal vein pressure values at each stage).



ular filtration down to the 'critical level' at which sodium is retained, about  $\frac{1}{2}$  to  $\frac{2}{3}$  of normal (10).

Our results agree with those of Bradley and his associates on human subjects in that the reduction in clearances is directly proportional to reduction in perfusion pressure across the renal circuit (P/F ratio remains constant). The finding of the constant filtration fraction is also confirmed. This signifies that there is no significant increase in intraglomerular pressure. The reduction in renal blood flow and clearances has been less than Bradley reports despite a slightly greater absolute elevation in renal vein pressure. However, this may be harmonized by considering the effect of venous pressure elevation in terms of total reduction in pressure gradient across the kidney (i.e., 10 and 13 per cent in the present data as compared with 18 per cent in the data of the human abdominal compression experiments).

#### SUMMARY

When renal venous pressure is elevated from 7.5 to 22.4 mm. Hg by partial venous obstruction, direct renal blood flow and renal clearances (PAH and creatinine)

<sup>2</sup>The possibility that blood flow might be maintained during graded renal vein obstruction by opening up of collateral venous channels does not seem very likely in acute experiments. When kidneys were perfused *in situ* in freshly killed dogs via the renal vein (artery occluded) at 30 mm. Hg pressure, the highest flow noted was 0.08 cc/min/gm. of kidney, a negligible figure.

decrease by an average of 15 per cent. This decrease in blood flow and clearances can be explained almost entirely by the decrease in pressure gradient across the renal vascular circuit resulting from increased venous pressure, since arterial pressure remains essentially constant. Thus, the A-V pressure difference decreases by an average of 11.5 per cent.

The clearances of PAH and creatinine show parallel reductions, hence there is no alteration in the filtration fraction attributable to venous obstruction. Therefore, no support can be given to the concept that elevated venous pressure increases intraglomerular pressure, at least not in the range studied. The conclusion follows that the increased filtration fraction noted in congestive heart failure is probably attributable to increased efferent arteriolar resistance rather than to high venous pressure.

The reduction in glomerular filtration rate resulting from experimentally elevated renal venous pressure is not enough to favor sodium retention and, in turn, edema formation. Other mechanisms must be operative in congestive heart failure.

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# ENHANCING EFFECTS OF GROWTH HORMONE ON RENAL FUNCTION<sup>1</sup>

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**W**E HAVE previously reported (1, 2) that the considerable falls in diodrast or sodium para-aminohippurate (PAH)<sup>2</sup> and inulin plasma clearances and in diodrast or PAH Tm resulting from hypophysectomy in dogs are not due to a depression of thyroid, of gonad or of adrenal cortical function brought about by the hypophysectomy; the question as to whether loss of growth hormone or of some further anterior-lobe principle is responsible for the falls was left unsettled. In the present paper it is shown that loss of growth hormone is responsible, since its administration to hypophysectomized dogs raises the depressed values to or above normal and to normal dogs raises normal values to twice the normal.

## METHODS

The clearance procedures and chemical methods were as previously described (2). Growth hormone prepared according to the Wilhelmi-Fishman procedure was generously supplied by the Armour Laboratories, Chicago. It was given daily subcutaneously for periods of 9 to 12 days. Except for the last 3 days with K44 we used Armour's lot 3PKR3, giving 0.5 mg/kg. daily. Armour Laboratories state that 50 gamma/rat/day of this material is sufficient to cause a 20 gm. weight increase in 15 days, that its adrenotropic and gonadotropic activities are negligible and that its thyrotrophic potency is estimated to be quite low. Published comparisons of the relative sensitivities of the dog and the rat to growth hormone are few. Putnam, Teel and Benedict (3) found that 1 to 2 cc/kg/day of their extracts produced increased growth in dogs, while increased growth in rats was produced by daily doses of up to 4 cc. per rat. Evans, Meyer and Simpson (4) found that 1 cc. of extract daily to adult female rats regularly stimulated growth of 55 gm. in 20 days. Daily dosage of 25 to 40 cc. of the same type of extract in a hypophysectomized puppy produced growth greater than that of a normal litter-mate control. The hypophysectomized puppy weighed 2.1 kg. at the beginning and 7.3 kg. at the end of the period of injections; it thus received 5 to 12 cc/kg/day as compared with 4 cc/kg/day for the rats. Normal dachshund pups receiving 0.5 to 1 cc. of extract daily grew much faster than litter-mate controls; shepherd pups were less responsive. It thus appears that the effective dose per kg. of dog approximates that per kg. of rat, with considerable variations in different breeds of dogs. Since 0.05 mg. of Armour's lot 3PKR3 is an effective dose in young rats, this is about 0.5 mg/kg of rat. This dosage was accordingly used in our dogs; it is not at all certain that maximum effects have been attained. For the last three injections on K44 we used Armour's lot 3PKS3R; the dose was 1.25 mg/kg/day, since it was stated to have approximately one half the potency of lot 3PKR3.

Received for publication January 10, 1949.

<sup>1</sup> Aided by grants from the Commonwealth Fund and from the U. S. Public Health Service RG-1070.

<sup>2</sup> The PAH was generously supplied by Sharp & Dohme, Philadelphia.

## RESULTS

*Normals.* The effects of growth hormone administration on the observed renal functions of 2 normal dogs are shown in table 1. It is seen that PAH and inulin clearances and PAH Tm are greatly increased after 9 or 12 days, although there is little or no effect after 5 days (K43-11/3/48). The values have begun to fall in 2 days and have returned to or slightly below normal by 6 days after cessation of growth-hormone injections.

*Hypophysectomized.* Table 2 shows that the depressed functions of hypophysectomized dogs are raised to or above normal after 9 days of daily growth-hormone administration; here an effect is apparent after 5 days (K39-11/29/48). The falls in plasma nonprotein nitrogen accompanying the clearance increases should be noted.

TABLE 1. EFFECTS OF GROWTH HORMONE ON RENAL FUNCTIONS IN NORMAL DOGS

		PAH CLEAR- ANCE	INULIN CLEAR- ANCE	PAH TM	PLASMA GLU- COSE
<i>Dog K43</i>					
10/28/48	Normal	352	116	20	81
10/29/48	through 11/10/48—0.5 mg/kg. growth hormone (3PKR <sub>3</sub> )				
11/3/48	5 days of growth hormone	336	117	27	81
11/10/48	12 days of growth hormone	849	170	33	91
11/12/48		608	138	20	79
11/16/48		308	91	16	80
<i>Dog K44</i>					
12/21/48	Normal	229	78	19	93
12/21/48	through 12/27/48—0.5 mg/kg. growth hormone (3PKR <sub>3</sub> )				
12/28/48	through 12/30/48—1.25 mg/kg. growth hormone (3PKS <sub>3</sub> R)				
12/30/48	9 days of growth hormone	388	124	35	117

Growth hormone daily subcutaneously.

*Adrenalectomized.* Table 3 shows that the response to growth hormone of the renal functions of a bilaterally adrenalectomized dog maintained on subcutaneously implanted desoxycorticosterone acetate (DCA)<sup>3</sup> pellets is slight or absent. There is a small increase after 5 days of injections (11/1/48) but this is not seen after 12 days (11/8/48). In an effort to discover whether the failure of the striking effects seen in the normal and the hypophysectomized dogs was due here to a deficit of adrenal cortical hormone (failure of DCA pellets to afford adrenal cortical replacement adequate for exhibition of growth-hormone effect) the experiment was repeated with the animal also receiving 0.2 cc. daily of Upjohn lipo-adrenal cortex intramuscularly. Here also no increase was obtained; the result is surprising in that there was an actual decrease of PAH clearance and Tm.

<sup>3</sup> The DCA pellets were generously supplied by the Schering Corporation, Bloomfield, N. J.

The failure of this dog to show the enhancing effects of growth hormone seen in normal and hypophysectomized dogs cannot be ascribed to a poor general condition due to adrenocortical deficiency; its appetite, weight, activity and disposition were normal, clearances and PAH Tm were within normal limits, and repeated plasma sodium and potassium values normal; e.g., on 11/24/48 plasma Na and K were 145 and 5.6 mEq/l., on 1/3/49 they were 143 and 5.0. The dog showed, however, the moderately

TABLE 2. EFFECTS OF GROWTH HORMONE ON RENAL FUNCTIONS IN HYPOPHYSECTOMIZED DOGS

		PAH CLEAR- ANCE	INULIN CLEAR- ANCE	PAH TM	PLASMA NPN	PLASMA GLU- COSE
<i>K<sub>39</sub></i>						
6/13/47	Normal	264	95	19		
6/16/47	Normal	263	96	23		
6/20/47	Simple hypophysectomy					
7/17/47		158	63	6.1		
9/10/47		176	44	8.8		
12/31/47		151	45	8.6		66
2/16/48		129	52	5.6	37	71
11/22/48		124	51	6.8		65
11/24/48	through 12/3/48—0.5 mg/kg. growth hormone (3PKR <sub>3</sub> )					
11/29/48	5 days of growth hormone	235	82	17	28	106
12/3/48	9 days of growth hormone	261	95	18		99
<i>K<sub>42</sub></i>						
11/10/47	Normal	247	77	22		
12/15/47	Normal	276	95	26		95
1/21/48	Simple hypophysectomy					94
2/26/48		130	53	5.1	66	92
3/24/48		124	49	6.5	50	80
12/7/48		149	54	9.3		82
12/7/48	through 12/16/48—0.5 mg/kg. growth hormone (3PKR <sub>3</sub> )					
12/16/48	9 days of growth hormone	353	110	26	27	121

Growth hormone daily subcutaneously.

elevated plasma nonprotein nitrogen values which we have consistently seen in DCA-supported adrenalectomized dogs even though they showed no other abnormality.

#### DISCUSSION

The demonstration that growth hormone raises the depressed renal functions of hypophysectomized dogs to or above normal and raises the values of normal dogs to twice the normal makes it unnecessary to postulate some further 'renotrophic' anterior-lobe principle whose loss is mainly responsible for the depression of renal functions resulting from hypophysectomy; our earlier demonstrations that loss of the gonado-

trophic and adrenotrophic hormones is not responsible and that the effect of loss of thyrotrophic hormone is slight (1, 2, 11) restrict the principal effect to loss of growth hormone or of some substance not separated from it. The results are consistent with the finding of an increased urea clearance in acromegaly (5). It may safely be accepted that the growth-promoting principle is formed by the eosinophiles (14); the concept that the principle responsible for the enhancing effects on renal function is formed by the eosinophiles is further supported by the finding that in Cushing's disease, in the absence of renal disease, with basophilic hyalinization which presumably indicates ineffective secretion of the basophile cells there is no depression of renal function (5), and by our earlier findings that dogs with denervated neurohypophysis, resulting in loss of basophiles in the anterior lobe (15), show no persistent depression of renal function (6, 7).

TABLE 3. EFFECTS OF GROWTH HORMONE ON RENAL FUNCTION IN AN ADRENALECTOMIZED DOG

	PAH CLEAR- ANCE	INULIN CLEAR- ANCE	PAH TM	PLASMA NPN	PLASMA GLU- COSE
<i>K<sub>41</sub></i>					
1/21/48			cc/min/ $M^2$	cc/min/ $M^2$	mg/ min/ $M^2$
Left adrenalectomy; 3 pellets (75 mg. each) desoxy-corticosterone acetate implanted					
1/28/48					
Right adrenalectomy					
2/24/48	213	86	12	37	75
10/25/48	276	96	14	46	85
10/27/48	through 11/8/48—0.5 mg/kg. growth hormone (3PKR <sub>3</sub> )				
11/1/48	5 days of growth hormone	313	110	17	115
11/8/48	12 days of growth hormone	205	76	16	91
11/12/48	through 11/24/48—0.5 mg/kg. growth hormone (3PKR <sub>3</sub> ) and 0.2 cc. lipo-adrenal cortex				
11/18/48	5 days of hormones	229	89	6.0	74
11/24/48	12 days of hormones	182	96	4.8	78

Growth hormone or growth hormone plus lipo-adrenal cortex daily.

The interpretation of the findings of table 3 is not clear. A possible interpretation is that the striking enhancing effects of growth hormone on renal function as seen in the normal and the hypophysectomized dog do not occur in the absence of some adrenal hormone which is not DCA and is not contained in adequate amount in our dosage of Upjohn lipo-adrenal cortex; it would seem more likely to be a cortical than a medullary product. An analogy may be drawn with the need for thyroid substance to obtain renal responses to anterior-lobe administration; Preloban produces considerable increases in normal dogs (11) but only slight and inconsistent increases in thyroidectomized dogs (1). However, the presence of DCA alone affords an adequate background to permit endogenous growth hormone to maintain normal renal function in the adrenalectomized nonhypophysectomized dog (1, 2) and to permit smaller and shorter lasting increases in response to Preloban, as in dog K<sub>31</sub> (1), and to growth hormone, as on 11/1/48 of table 3. A further possibility is that smaller doses of

lipo-adrenal cortex would have made possible the full enhancing effects of growth hormone and that the dosage employed was high enough to achieve an effective antagonism between adrenocortical and growth hormones such as has been described for the growth-promoting effect (16). Some support to the view of antagonism may be afforded by the observations that whereas growth hormone raised the fasting plasma glucose levels in the normal and hypophysectomized dogs (tables 1 and 2) and in the adrenalectomized dog supported with DCA alone, it did not when lipo-adrenal cortex was added.

Acute renal hyperemia has been produced by pyrogens, by intravenous amino acid and by intravenous adenine derivatives (8). Chronic renal hyperemia has been produced by high-protein diet in dogs (9) and less strikingly in man (10); it also results from thyroid or anterior lobe administration (1, 11-13). Our present finding of a non-toxic substance which does not raise general metabolic rate or body temperature and which produces striking chronic increases in renal blood flow, filtration rate and tubular activity may be clinically useful in certain conditions where such effects may be desirable.

#### SUMMARY

Daily growth-hormone administration for 9 to 12 days doubles the PAH clearance and almost doubles the inulin clearance and PAH Tm in normal dogs and raises the greatly depressed values of hypophysectomized dogs to or above the normal levels. It has but slight effect on these functions in the adrenalectomized dog maintained on DCA pellets. In view of our earlier demonstrations that loss of the gonadotrophic and adrenotrophic hormones is not responsible for the great depressions of these renal functions seen after hypophysectomy, and that the effect of loss of thyrotrophic hormone is slight, the principal effect is due to loss of growth hormone or of some substance not yet separated from it. The enhancing effect of growth hormone on renal function may find some therapeutic application.

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# COMPARISON OF THE CARBOHYDRATE EFFECTS OF ADRENALIN INFUSED INTO THE FEMORAL VEIN, CAROTID ARTERY, AORTA AND PORTAL VEIN OF RATS<sup>1</sup>

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THE effects of adrenalin introduced into a peripheral vein on the distribution of body carbohydrate are well known. The changes produced by adrenalin when infused into other vessels have been poorly studied. This is particularly true of the effects on carbohydrate metabolism, although the circulatory changes have been well observed (1-3). Intra-arterial adrenalin causes circulatory disturbances in the related limb, but general effects are minimal. It is postulated that adrenalin is rapidly destroyed in the tissues, and when introduced into a limb artery, very little passes into the general circulation.

In the present study normal rats were perfused with adrenalin into the femoral vein, the aorta (thoracic and abdominal), and the carotid artery. The substance was also perfused into the portal vein to determine the direct effects on the liver. The changes produced in liver glycogen and blood sugar levels and in muscle glycogen and blood lactic acid were noted.

## EXPERIMENTAL

Adult male rats of the Sprague-Dawley strain were used. They were fed in *ad libitum* diet of Purina Laboratory Chow with Viobin corn germ supplement and were fasted for 48 hours previous to the experiment.

The infusions were made through a no. 26 hypodermic needle cemented into a No. 4 French ureteric catheter. This was attached to a 2-ml. syringe, the plunger of which was driven at a constant rate by an electric pump (manufactured by the Johnson Foundation, University of Pennsylvania).

Adrenalin hydrochloride solution (1:1,000 Parke Davis) was diluted 1:250 or 1:500 with 0.9 per cent sodium chloride solution, and glutathione (2 mg/10 ml. infusion fluid) was added. Heparin (one drop to 10 ml. solution) was included for the intra-arterial infusions. The solution was infused for 60 minutes, a total volume of 1.6 to 1.8 ml. being given. The dosage was approximately  $3 \times 10^{-5}$  mg/100-gm. rat/minute.

The rats were anesthetized with intraperitoneal Nembutal solution in a dose of 5 mg/100-gm. rat. Blood samples of 0.2 ml. were taken from the tail, and the infusion was then started and allowed to run for one hour. A further blood sample

Received for publication December 26, 1948.

<sup>1</sup> This investigation was aided by a grant from the James Hudson Brown Fund.

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was then taken, and the gastrocnemius muscle removed for analysis. The abdomen was then opened and the left lobe of the liver removed for glycogen analysis. Body temperature was maintained during the observation by an electric bulb placed over, but not too close to the rat. The experiments were usually done in pairs on the same day; in one animal adrenalin was infused while in the other control observation 0.9 per cent sodium chloride solution was infused for one hour.

*Femoral Vein Infusions.* These infusions were made directly into the exposed vein.

*Carotid Artery Infusions.* With the head extended, a midline cervical incision was made through skin and fasciae and the left sternomastoid muscle retracted laterally. The carotid sheath of vessels was exposed and more fully visualized by incision of the omohyoid muscle as it crosses these vessels. The carotid artery being isolated, a cotton suture was threaded under it and used as a retractor. The needle was inserted in a cephalic direction, and the infusion commenced. The wound was covered with a gauze swab moistened with warm normal saline.

*Aortic Infusions: Abdominal Aorta.* A lower midline abdominal incision was made. The intestines were displaced to the right, and the aorta exposed by a small incision through the peritoneum of the posterior abdominal wall. A point was chosen in the aorta below the renal artery, and the needle rapidly inserted in a cephalic direction. In a few instances this procedure resulted in some hemorrhage; these experiments were discarded. The infusion was started and the skin and muscles of the abdominal wall brought together by a skin clip. The wound was covered with a gauze swab moistened with saline.

*Aortic Arch.* The abdominal aorta was exposed as described above. A small 'bull dog' clamp was applied to the aorta just below the renal artery. The aorta was now ligatured just above its point of division into the common iliac arteries. A further loop-ligature was passed around the aorta. A slit was made in the aorta just proximal to its ligature, and a fine Vinylite tube inserted. The 'bull dog' clamp was removed and the tube pushed along the aorta for a distance of approximately 9 cm. At this distance a slight resistance was encountered, and subsequent dissection showed that the tip of the tube rested in the arch of the thoracic aorta. The tube is kept in position by tightening the loop ligature. If the manipulations are rapid, there is surprisingly little bleeding. The tubing is attached to the syringe, and infusion of adrenalin or of saline is started. It is thus possible to infuse the solution, against the direction of blood flow, directly into the aortic arch.

The blood supply to the lower limbs is greatly altered by ligature of the aorta. Due to the anoxic conditions in the lower limb, analysis of the tail-blood sample showed a conspicuous rise in lactic acid and fall in sugar levels. In these experiments, therefore, the final blood sample was taken from the heart.

Blood glucose was determined by the Nelson (4) method and blood lactic acid by the method of Barker and Summerson (5). Hepatic and muscle glycogen values were estimated by a modification of the Good, Kramer and Somogyi procedure (6).

## RESULTS

*Femoral Vein Infusion.* Compared with the control group, adrenalin infusions resulted in a conspicuous rise in blood glucose and lactic acid levels. Hepatic glyco-

gen increased, and muscle glycogen diminished. Doubling the concentration of adrenalin resulted in an approximately two-fold rise in blood glucose, but the blood lactic acid level showed no significant difference from the smaller dose. The muscle glycogen values were, however, lower. The rise in liver glycogen is presumably related to the deposition of lactic acid released from muscle.

*Carotid Artery Infusions.* The control group in this series showed greater changes than controls given saline intravenously. The operative procedure had, therefore, a greater general effect than infusion of saline into the femoral vein. The infusion of adrenalin resulted in a slight increase in blood glucose and lactic acid concentrations. Muscle and liver glycogen values showed no conspicuous change. The findings do not support the postulate that the effects of adrenalin on carbohydrate metabolism are primarily mediated through any structure in the vascular territory of the carotid artery.

*Portal Vein Infusions.* The control observations show that saline infused into the portal vein results in a slight increase in blood glucose levels. Muscle glycogen and blood lactic acid values are not altered. When adrenalin is infused, there is a slight but insignificant rise in blood sugar. Hepatic and muscle glycogen and blood lactic acid are not significantly altered. However, as the liver glycogen levels in both the test and control groups were rather low, the experiments were repeated on animals previously fed glucose (table 1). This resulted in higher liver glycogen values. The results obtained were essentially similar to those found when the hepatic glycogen levels were low.

*Aortic Infusions: Abdominal Aorta.* Control observations show that the operation alone with infusion of saline produced little change in the distribution of carbohydrate. The infusion of adrenalin into the abdominal aorta resulted in only slight glycemia. The change in hepatic glycogen was not significant. However, there was a conspicuous rise in blood lactic acid values and a fall in the level of muscle glycogen. The adrenalin had therefore resulted in glycogenolysis in muscle. Almost identical results were obtained when the dose of adrenalin was halved.

*Aortic Arch Infusions.* Although adrenalin infused into the portal vein produced little effect on liver glycogen, it seemed possible that infusion into the hepatic artery might be effective. This procedure would offer great technical difficulties in the rat. However, infusion into the thoracic aorta would indirectly perfuse the hepatic artery through the coeliac axis. When this was done, the changes in blood glucose were not so great as perfusion into the femoral vein. The hepatic glycogen values were not significantly different from those of control values. Ligation of the abdominal aorta necessarily diminishes the vascular bed of the body. When this is taken into consideration, it seems that there is little difference between the changes in liver glycogen and blood glucose values produced by infusion of adrenalin into abdominal or thoracic aorta or into the portal vein. Aortic arch infusion, however, produces very complex changes in the distribution of body carbohydrate. The anoxic state of the lower limbs has already been mentioned; the low muscle glycogen values in both test and control values reflects this anoxia. However, the obstruction produced by the Vinylite tubing to blood flowing down the aorta was probably not very great. Histological sections of liver taken from control group animals at the conclusion of the experiment

TABLE I. EFFECT OF ADRENALIN INFUSED INTO FEMORAL VEIN, CAROTID ARTERY, ABDOMINAL OR THORACIC AORTA, AND PORTAL VEIN ON BLOOD GLUCOSE, BLOOD LACTIC ACID AND LIVER AND MUSCLE GLYCOGEN LEVELS

SITE OF INFUSION	SOLUTION INFUSED	CONCENTRATION	NO. RATS	BLOOD GLUCOSE	BLOOD LACTIC ACID	GLYCOGEN %
				MG/100 ML.	MG/100 ML.	
				Control	Change	
Femoral vein	Saline		6	76 1.1 ± 2.6 <sup>1</sup>	23.6 -7.2 ± 1.1	387 ± 504 ± 22.4 26.9
	Adrenalin	1:500,000	6	76 37.0 ± 2.8	26.8 8.3 ± 2.6	870 ± 415 ± 65.2 14.2
	Adrenalin	1:250,000	7	72 74.3 ± 8.8	23.9 10.3 ± 3.4	477 ± 318 ± 28.1 20.7
Carotid artery	Saline		6	71 7.3 ± 3.3	19.3 -3.1 ± 1.7	489 ± 508 ± 48.2 17.2
	Adrenalin	1:250,000	7	71 30.0 ± 2.9	21.2 3.1 ± 0.8	492 ± 449 ± 31.9 16.7
	Adrenalin		5	70 3.6 ± 3.1	21.9 -7.0 ± 0.8	442 ± 469 ± 51.8 19.8
Abdominal aorta	Adrenalin	1:500,000	6	73 16.0 ± 4.2	26.6 13.7 ± 5.4	466 ± 313 ± 42.1 22.3
	Adrenalin	1:250,000	6	74 17.0 ± 5.3	23.6 10.0 ± 2.2	322 ± 326 ± 11.3 23.2
	Saline		5	65 3.8 ± 1.0	21.9 -12.6 ± 2.2	348 ± 253 ± 22.8 39.1
Arch of aorta	Adrenalin	1:250,000	6	66 37.1 ± 4.7	19.9 -6.0 ± 0.7	274 ± 269 ± 26.5 23.8
	Saline		5	64 5.8 ± 2.1	20.9 -8.2 ± 0.6	188 ± 515 ± 24.2 20.4
	Adrenalin	1:250,000	6	66 17.3 ± 2.8	21.9 -8.1 ± 1.1	218 ± 485 ± 19.8 18.7
2	Saline		5	83 7.6 ± 2.2	21.7 -4.5 ± 0.7	1613 ± 605 ± 43.8 44.5
	Adrenalin	1:250,000	5	88 13.0 ± 3.5	24.8 -7.2 ± 1.0	1598 ± 572 ± 52.8 40.5

<sup>1</sup> Mean ± standard error of mean. <sup>2</sup> Animals given 5 ml. of 50% glucose solution by stomach tube 18 hours before the experiment.

showed no significant pathological changes. Any profound disturbance of the hepatic circulation, therefore, seems unlikely.

#### DISCUSSION

The infusion of adrenalin into a peripheral vein produced changes in carbohydrate distribution compatible with those of other workers using similar dosage (7). However, when adrenalin was introduced into an artery or into the portal vein, quite different changes were produced. The substance was now delivered directly to the organ concerned. Adrenalin is destroyed by tissues very rapidly and remains for only a short time in the blood stream. The liver probably has the greatest power of inactivation (8, 9). Intraportal or intra-arterial adrenalin would be expected to cause changes in the territory of the vessel infused. The substance would then be destroyed in the tissues, and if the dose was not excessive, general effects would be minimal.

In the case of the abdominal aorta, the above sequence of events apparently occurred. A direct action on the lower limb muscles resulted in glycogenolysis and consequent increase in blood lactic acid concentration. The adrenalin is infused only into the lower limb muscles, and the changes recorded in muscle are therefore more conspicuous than when the same dose is given intravenously. The slight rise in blood sugar is attributed to failure of tissue destruction of all the adrenalin, some passing into the general circulation. These direct effects of adrenalin on skeletal muscle in the rat are contrary to those described for man. The infusion of adrenalin into the femoral artery of man produces no change in the glycogen content of the ipsilateral gastrocnemius muscle, and the lactic acid content of femoral vein blood from the same limb is not increased (10). This discrepancy is not explainable on the basis of dosage. The dose used in man, after one hour's intravenous infusion, produced a rise in blood sugar of 52 mg/100 ml. (11). This compares well with the blood sugar changes recorded with intravenous infusion in the rat. Borysiewicz (12) injected adrenalin into the femoral artery of dogs and found a normal glycemic response but no change in blood pressure. Baudouin and coworkers (13) also in dogs, using the infusion technique, report that twice to four times the intrafemoral arterial dose of adrenalin was needed to produce the same glycemia as when given intravenously. In neither of these papers were the blood lactic acid and muscle glycogen values recorded.

The failure of adrenalin introduced into the portal vein to discharge liver glycogen, even when hepatic glycogen stores were adequate, was most unexpected. As adrenalin is readily destroyed by the liver, there was little effect on muscle glycogen. In dogs Borysiewicz (12) reported that the blood sugar rise is identical, whether the adrenalin is given into a peripheral or mesenteric vein, whereas Baudouin *et al.* (13) showed that twice the dose of intramesenteric venous adrenalin was needed to produce the same rise of blood sugar as when given into a peripheral vein. Perfusion of the isolated dog's liver with adrenalin is known to cause glycogenolysis (14, 15). None of these observations is readily comparable with the present work, the species used, the type of anesthesia, the techniques of administration, and the dosage of

adrenalin being very different. Circulating adrenalin normally reaches the liver through the hepatic artery. However, mixing with blood occurs in the hepatic sinu-soids, and it is with sinusoidal blood that the individual liver cells come in contact. However, infusion of adrenalin into the aortic arch so that the liver cells received the substance *per via naturalis* also failed to cause glycogenolysis. It seems that in the rat adrenalin does not exert a direct effect on liver glycogen. It might be postulated that the liver is affected secondarily to the changes in muscle. The failure of intra-aortic infusions to discharge liver glycogen even when the glycogenolysis in muscle is vigorous makes this unlikely.

Adrenalin is believed to activate the anterior pituitary to release adrenocorticotrophic hormone (16). As adrenalin infused into the portal vein failed to directly affect liver glycogen, it seemed possible that some effects might be mediated through the central nervous system or pituitary and adrenal cortex. However, infusion of the carotid artery with presumably delivery of a large amount of adrenalin directly to the brain failed to produce the effects obtained by infusion into the femoral vein. Moreover, the glycemic response to intravenous adrenalin is normal in hypophysectomized rats (7). The diminished metabolic response to adrenalin when the substance is infused into the carotid artery is probably due to its destruction in the tissues of the head and neck, very little passing into the venous side to affect liver and muscle glycogen.

#### SUMMARY

Adrenalin infused into the femoral vein of adult male rats causes a diminution in muscle glycogen, an increase in blood lactic acid and blood glucose levels, and an increase in hepatic glycogen concentration. Adrenalin infused into the abdominal aorta caused a depletion of the glycogen of the gastrocnemius muscle with a rise in blood lactic acid, but little change in blood sugar or hepatic glycogen. Adrenalin infused into the arch of the aorta resulted in only slight glycemia with little effect on liver glycogen. Adrenalin similarly infused into the portal vein was without effect on hepatic glycogen, lactic acid, or muscle glycogen. The increase in blood sugar was small. Intra-carotid artery infusions of adrenalin resulted in only slight hyperglycemia and lactacidemia with no significant change in muscle or liver glycogen.

Adrenalin introduced into an artery is believed to be rapidly destroyed by the tissues into which it is delivered. It exerts a direct glycogenolytic effect on muscle but in the rat apparently has no direct effect on liver glycogen.

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# EFFECT OF HORMONES OF THE POSTERIOR PITUITARY ON TOLERANCE OF THE EVISCERATED RAT FOR GLUCOSE

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**S**TUDIES from this laboratory (1) have shown that epinephrine can affect the glucose tolerance of the eviscerated rat and that the nature of the response is related to the experimental conditions. When glucose without insulin was administered to eviscerated rats, the addition of epinephrine did not affect glucose tolerance during the first 2 hours but during 24 hours there was an increase in glucose requirement. When glucose with insulin was administered to eviscerated rats, the addition of epinephrine caused a decrease in glucose tolerance within 2 hours which continued throughout 24 hours. In the present studies it was found that an extract of posterior pituitary affected glucose tolerance in the same manner as did epinephrine. A more highly purified preparation of the pressor principle of the posterior pituitary had similar effects and a preparation of the oxytocic principle which was not free from pressor activity had a relatively weak effect upon glucose tolerance when studied during a period of 2 hours.

## METHODS

Male rats of the Sprague-Dawley strain were fed Archer Dog Pellets. At a weight of 185 to 205 grams, the inferior vena cava was ligated between the liver and kidneys in order to cause the development of a collateral circulation. Asepsis was preserved in this operation. When the animals reached a weight of 250 ( $\pm 2$ ) grams they were anesthetized (intraperitoneal injection of 18 mg. of cyclopentenyl-allyl-barbituric acid sodium) and eviscerated by the procedure of Ingle and Griffith (2).

Solutions containing 0.9 per cent sodium chloride and varying concentrations of glucose (C.P. Dextrose, Merck) with and without regular insulin (Lilly) (4 U/24 hr./rat) were infused into the saphenous vein of the right hind leg by means of a constant injection machine which delivered fluid from each of 6 syringes at the rate of 20 cc. in 24 hours. The glucose load is expressed as milligrams of glucose per 100 grams of rat per hour (mg/100/hr.). The infusions covered periods of 2 and 24 hours. Analyses of glucose by the method of Miller and Van Slyke (3) were made on jugular vein blood taken at the end of the infusion periods.

The extracts of posterior pituitary were added to solutions of glucose and insulin which were given intravenously. The preparations tested were posterior pituitary extract (Upjohn) 20 I.U. per cc.; pressor principle (Pitressin, Parke, Davis) 20 pressor units per cc.; and oxytocic principle (Pitocin, Parke, Davis) 10 I.U. per cc.

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Received for publication January 14, 1949.

## EXPERIMENTS AND RESULTS

*Experiment 1* (fig. 1) was a study of the effect of posterior pituitary extract (Upjohn) upon the glucose tolerance of the eviscerated rat. Twelve pairs of rats were represented in each group.

Three groups of rats were given a glucose load of 64/100/hr. with insulin for a period of 2 hours. Dilutions of posterior pituitary extract of 1, 2 and 4 parts per 100 each caused a significant rise in the level of blood glucose as compared to that of the control animals. Two groups of rats were given a glucose load of 16/100/hr. without insulin for a period of 2 hours. Dilutions of posterior extract of 1 and 2 parts per 100 had no significant effect upon the level of blood glucose. Two groups of rats were given a glucose load of 40/100/hr. with insulin for a period of 24 hours. Dilutions of posterior pituitary extract of 1 and 2 parts per 100 each caused a significant rise in the level of blood glucose as compared to that of the control animals.

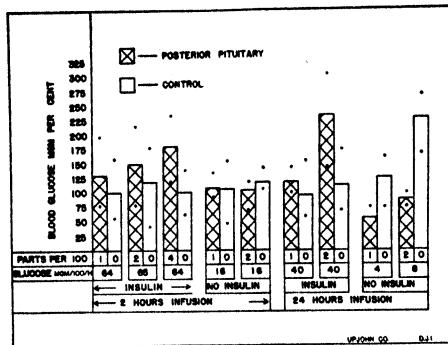


Fig. 1. EFFECT OF POSTERIOR PITUITARY EXTRACT upon the level of blood glucose at the end of the infusion period. Averages and range. Twelve rats per group.

One group of rats was given a glucose load of 4/100/hr. without insulin for 24 hours. A dilution of posterior pituitary extract of 1 part per 100 caused a significant decrease in the level of blood glucose as compared to that of the control animals. A second group of rats was given a glucose load of 8/100/hr. without insulin for 24 hours. A dilution of posterior pituitary extract of 2 parts per 100 caused a significant suppression of the blood glucose level as compared to that of the control animals.

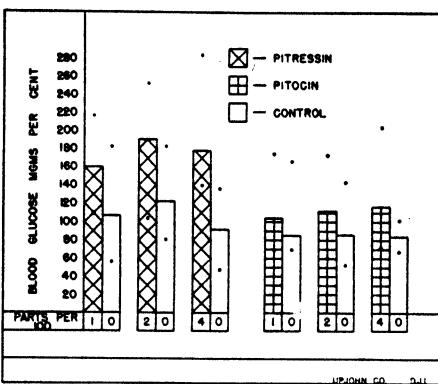
*Experiment 2* (fig. 2) was a study of the effect of the pressor and oxytocic principles upon the glucose tolerance of the eviscerated rat. Twelve pairs of rats were represented in each group.

Three groups of rats were given a glucose load of 64/100/hr. with insulin for 2 hours. Dilutions of pressor principle of 1, 2 and 4 parts per 100 each caused a significant rise in the level of blood glucose as compared to that of the control animals. Three groups of rats were given a glucose load of 64/100/hr. with insulin for 2 hours. Dilutions of the oxytocic principle of 1, 2 and 4 parts per 100 each caused a small but definite rise in the level of blood glucose as compared to that of the control animals. After demonstrating that the preparation of the oxytocic principle had an effect upon glucose tolerance similar to that of the pressor principle, this preparation was assayed for its effect upon the blood pressure of the cat. According to this criterion the prep-

aration of oxytocic principle used by us contained approximately 20 per cent as much pressor principle per unit volume as did the preparation of the pressor principle.

Each of the 3 preparations of posterior pituitary hormones used in this study contained 0.5 per cent of chlorobutanol as a preservative. Twenty-four pairs of eviscerated rats were given 64/100/hr. of glucose with insulin for 2 hours. The infusion fluid for one rat of each pair contained chlorobutanol in a concentration of 0.02 per cent which equaled the highest concentration in the dilutions of posterior pituitary preparations. The average blood glucose value for the rats receiving chlorobutanol was 104 mg. per cent as compared to the average of 111 mg. per cent for the control animals. It was therefore concluded that chlorobutanol was not the cause of

Fig. 2. EFFECT OF THE PRESSOR AND OXYTOCIC FRACTIONS OF POSTERIOR PITUITARY EXTRACT UPON THE LEVEL OF BLOOD GLUCOSE AT THE END OF TWO HOURS OF INFUSION. AVERAGES AND RANGE. TWELVE RATS PER GROUP. GLUCOSE LOAD OF 64/100/HR. WITH INSULIN.



the rise in blood glucose when posterior pituitary preparations were administered under identical conditions.

#### DISCUSSION

These studies show that posterior pituitary extracts affect the glucose tolerance of the eviscerated rat in the same manner as does epinephrine (1). When glucose without insulin was administered to eviscerated rats for a period of 2 hours, the addition of posterior pituitary extract did not have any significant effect upon the level of blood glucose but during a period of 24 hours the addition of posterior pituitary extract caused a significant suppression of the level of blood glucose. When glucose with insulin was administered to eviscerated rats, the addition of posterior pituitary extract caused a significant rise in the level of blood glucose during periods of 2 and 24 hours.

The preparations of pressor and oxytocic principles were tested only in eviscerated rats given a glucose load of 64/100/hr. with insulin for a period of 2 hours. The preparation (Pitressin) which was high in pressor activity caused a marked rise in blood glucose just as did epinephrine (1) and posterior pituitary extract (*exp. 1*). The preparation of oxytocic principle (Pitocin) had a less marked effect which roughly paralleled the extent of its contamination with the pressor principle.

The mechanism whereby these principles which act upon smooth muscle also

affect glucose tolerance is not known to us. The problem is made more complex by the fact that direction of the response is reversed by the presence or absence of insulin. Also, it is not known whether the effects of these hormones upon glucose tolerance in the eviscerated rat represent physiological mechanisms of action or whether they should be considered as pharmacologic responses.

#### SUMMARY

Eviscerated rats were infused intravenously with glucose with and without insulin for periods of 2 and 24 hours. When glucose without insulin was given, the addition of posterior pituitary extract did not have a significant effect upon glucose tolerance within 2 hours but during a 24-hour period there was a significant suppression of the level of blood glucose. When glucose with insulin was given, the addition of posterior pituitary extract caused a significant rise in the level of blood glucose during periods of 2 and 24 hours.

Preparations of the pressor and oxytocic principles with insulin were tested in eviscerated rats during a period of 2 hours. The preparation of the pressor principle caused a marked rise in blood glucose. The preparation of the oxytocic principle had a less marked effect which roughly parallel the extent of its contamination with the pressor principle.

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# EFFECT OF METABOLIC INHIBITORS ON MEMBRANE POTENTIALS IN THE SYNOVIALIS OF THE DOG<sup>1</sup>

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A PREVIOUS report (1) on the electrochemical properties of synovial tissues was concerned with the diffusion potentials observed with many ions relatively inactive with respect to tissue metabolism. The evidence for their inactivity included the agreement within reasonable limits of the observed membrane potentials with those estimated from the aqueous mobilities of the ions. The calculations were based on the theories of Planck (2) and of Henderson (3) on the nature of potentials at diffusion boundaries. In agreement with theory were the potentials of the alkali and alkaline earth chlorides, the potentials of NaCl in relation to its concentration, and the potentials of bromide, sulphate, salicylate and benzoate ions. Among the halogens, only iodide ion was found to deviate greatly from the potential estimated from its aqueous mobility. These results, with those on thiocyanate and some salicylate derivatives, appear to indicate, at least in part, metabolic reactivity rather than diffusion effects.

The investigation has been continued with the purpose of revealing the effect on the membrane potential of substances which have well-defined effects on metabolism such as inhibitors or activators of various types. By altering in a known manner the pathways of oxidation-reduction processes through known enzyme systems, such as the Warburg-Keillin system (cytochrome-cytochrome oxidase), the relation of the processes to the observed potentials may be determined. Conversely, unknown or hypothetical metabolic processes may be found, and their relative importance established by reference to the membrane potential as a criterion. It is our purpose to determine the nature of the relation of the membrane potential to metabolism.

## EXPERIMENTAL

The method of determining the synovial membrane potential in the knee joint of dogs has been described (1). A slight modification has been introduced to permit duplicate determinations on both knee joints with one pair of saturated KCl-calomel electrodes, rather than the two pairs of Ag-AgCl electrodes previously employed. This procedure eliminates the need of correcting small errors due to differences in the reference electrodes. The indicator electrode was connected to approximately isotonic (0.15 M) solutions within the joint cavity by means of the technique previously described. By using an extension bridge of saturated KCl, this electrode

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Received for publication December 28, 1948.

<sup>1</sup> The expenses of this investigation were borne by the Arthritis Research Project of the University of Illinois. A preliminary report was presented before the American Physiological Society, Minneapolis, September 17, 1948.

was connected to the solution being observed in either joint. The subcutaneous needle was filled with NaCl solution (0.15 M), and connected to a syringe barrel containing the same solution. These were connected to the reference calomel electrode by means of a saturated KCl bridge. In most experiments the procedure was to vary the solutions within one joint cavity, making EMF readings with every solution with time. The other joint was used as a control with NaCl (0.15 M). Later the observations were repeated on the control joint.

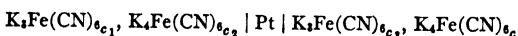
With a given solution, the fluid was aspirated and replaced a sufficient number of times to yield fairly stable, reproducible potentials. With numerous reagents, drifting potentials were obtained, but these usually reached a fairly steady state, often passing through a maximum or minimum value near which the potentials were stable. The behavior of these potentials was roughly characteristic of each inhibitor or activator. The most reproducible features were the levels at which the initial potentials appeared, the levels at maxima or minima, and the final stable levels which developed after long exposure. Differences of these levels between various inhibitors were generally of a larger order of magnitude than the range of potentials over which any drift occurred.

All inhibitor or activator solutions were made up to isotonic strength with NaCl. The sodium salts of the following substances were included: cyanide, sulphide, azide, malonate, o-iodobenzoate, iodoacetate, thioglycolate, fumarate, succinate, iodide, and thiocyanate. Cations included ferric, cupric, mercuric and hydroxylamine, introduced as the chlorides. Neutral substances included thiourea, urea, thioracil, hydrogen peroxide and molecular iodine. The effects of methylene blue also have been observed.

The experimental results are presented in tables 1 to 5, in which the reagents are classified according to the following types: 1) inhibitors of cytochromeoxidase; 2) thiols; 3) heavy metals and other sulphydryl inhibitors; 4) inhibitors and activators of succinic dehydrogenase; and 5) electron acceptors from cytochromeoxidase. Of course, most active substances fall into more than one group. Regardless of this fact, the classification is convenient and a useful first approximation, but it represents what appears to be the most predominant effect of the substance rather than the totality of its effects. The potentials which are given represent the averages selected according to some characteristic indicated in the tables. These are usually the initial potentials, or the most stable final potentials, depending on which characteristic is the more reproducible.

*Theoretical Considerations.* The membrane potentials which have been observed fall into two distinct classes. Those produced by the alkali and alkaline earth cations, by chloride, bromide, sulphate and other anions, are of the order of plus or minus 10 millivolts or less, compared with NaCl at isotonic concentrations. They are of the sign and magnitude predicted by the Planck-Henderson theory of diffusion potentials taking accepted values of the relative ionic mobilities and Hittorf transference numbers, as determined from conductance and transference data in aqueous solution (1). The potentials produced by cyanide, sulphide, ferric, cupric and other ions, known to act as metabolic inhibitors or activators, are of a much larger order of magnitude, greater than 400 millivolts in some cases, even at low concentrations compared with

the isotonic NaCl present in the solutions. The theory of diffusion potentials does not account for potentials of that order of magnitude under the conditions of the experiments. These potentials are to be classed as metabolic potentials, as distinguished from the diffusion potentials observed with metabolically inactive ions. In every case both factors, ionic mobility and metabolic effect, contribute to the observed potential, but the metabolic effect, when present, results in a potential that may be of a greater order of magnitude. In order to relate the experimental results to known effects of the inhibitors and activators, the following considerations are presented. Boundaries between two oxidation-reduction systems may be imagined to approach either of two limiting conditions: 1) boundaries at which occur oxidoreductions involving electron exchange but not ionic transference; and 2) those at which ionic transference takes place, but not electron exchange. The first will be termed 'boundaries without transference'. This type is exemplified by the model



in which platinum is the only connection between the solutions. When the potential difference is measured with a pair of identical KCl calomel electrodes connected to the two solutions, the electrochemical process is the reduction of ferricyanide and oxidation of ferrocyanide in the solution of lower redox potential. In the other solution the reaction goes in the opposite direction. The electromotive force,  $E$ , is given by the relation

$$E = \frac{RT}{F} \ln \frac{a_1 a_4}{a_2 a_3}. \quad (1)$$

$R$  denotes the gas constant,  $T$  the absolute temperature, and  $F$  the Faraday electrochemical equivalent, 96,500 coulombs; while  $a$  denotes the thermodynamic activity of an ion at the concentration  $c$ . In other words, the boundary potential can be calculated as the difference between the redox potentials of the two solutions measured individually. Thus

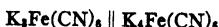
$$E = E_t - E_s = E_{st} \quad (2)$$

where  $E_s$  and  $E_t$  are the potentials at the electron source and terminal, respectively. The former represents the redox potential of the reducing solution and the terminal potential refers to that of the oxidizing solution.

The mechanism by which electrochemical oxidoreductions in tissues occur with electron exchange at boundaries will be assumed to be that of aerobic respiration over the cytochromes. Within recent years it has been generally accepted that this process involves the alternate oxidation and reduction of the individual ferro- and ferri-porphyrin enzymes, whereby electrons originating in the substrate first reduce one of the acceptor components, eventually reducing ferri-cytochrome oxidase, the respiratory enzyme of Warburg, and being accepted by molecular oxygen (4). Unlike many other enzymes, the cytochromes with the exception of the component  $c$  are quite insoluble. All occur predominantly as part of the insoluble structures of cells and tissues. The process of aerobic respiration over the system can be regarded, accordingly, as a mechanism involving the conduction of electrons from substrate to

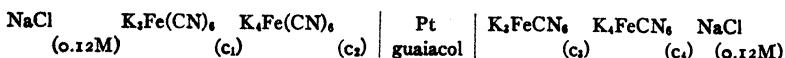
oxygen rather than as one in which the iron enzymes migrate. Szent-Györgyi (5) has described this as a series of quantum jumps, involving definite energy levels in the cytochrome system, and producing corresponding quanta of radiant energy. A somewhat related conception of electron conduction in other iron porphyrins has been developed (6). With the cytochrome system as conductor, a biological phase boundary at which tissue respiration occurs, can be represented as a boundary without transference analogous to the model. The electron source is the substrate, yielding electrons to cytochrome *b*, *c* or *a* in order of increasing standard potentials. The terminal is the oxygen- ferro- ferricytochrome oxidase system. The source, cytochrome *b*, for example, is represented in the model by ferrocyanide, and the terminal, cytochrome oxidase, by ferricyanide. The platinum conductor corresponds to the electron conducting system, represented in the tissues by the cytochromes acting as a whole.

As a next approximation the other limiting case, that of ionic transference, is considered. A 'boundary with transference' is exemplified by the model



where the double vertical lines represent an unspecified type of boundary between the oxidized and reduced ions. Processes involving electron exchange independent of ionic diffusion are excluded. The potential at such a boundary may be one of several types: diffusion (2,3); oil phase (7), or membrane with selective permeability (8). In any case the potential is determined primarily by the mobilities or transference numbers of the various ions without regard to the redox potentials. The electrical resistance at such a boundary is low in the case of a diffusion boundary, high in the case of an oil-phase boundary.

An electrochemical model applicable to tissues should include the properties of both models, with and without transference. Such a boundary will be termed a 'boundary with partial transference.' In particular it will be considered to have a high resistance. Earlier measurements have indicated the resistance of the synovial membrane to be of the order of 100,000 ohms (1). To illustrate some of the properties of the boundary with partial transference, the potentials of the following system were determined:



The two solutions were separated by a layer of guaiacol of variable thickness. They were connected by a platinum wire, serving as electron conductor. The potential was measured by means of identical saturated KCl calomel electrodes making liquid junctions with the two solutions. The ratio of concentrations c<sub>1</sub> to c<sub>2</sub> was 10.0 and that of c<sub>3</sub> to c<sub>4</sub> was 0.1. The total concentration of ferro- plus ferricyanide was 0.02 M in each solution. The theoretical EMF of the boundary without transference at 25°C. is

$$E = 0.05915 \log \frac{c_1 c_4}{c_2 c_3}$$

$$= 118.3 \text{ millivolts}$$

where concentrations are used instead of activities. This is approximately correct, because the ionic strengths of the two solutions are very nearly the same.

Measurements of this system gave 116 mv. for a thick guaiacol layer. As the thickness of the layer was continuously decreased, the potential difference fell continuously to a value of approximately 50 mv. for a thin film of guaiacol, at which point the potential broke and approached zero. For a cellophane membrane of much lower resistance, the potential difference was 3 mv. Thus the potential difference of a boundary with partial transference can be made to approach that of a boundary without transference when the conduction is made vanishingly small. For this case equation 2 determines the boundary potential.

In applying these principles to the experimental results, the following definitions are introduced. The mean source potential,  $\bar{E}_s$ , is defined as the mean potential at which electrons are accepted by the cytochromes from substrate via enzymes and carriers. At a given instant it is the potential averaged over all electrons, each of which is assumed to enter the cytochrome system at a potential which varies from point to point and from instant to instant. The mean is taken over all acceptor cytochromes, normally b, c, and a.  $\bar{E}_s$  is related to  $E_s$ , the source potential at a point by the relation

$$n_1 \bar{E}_s = \int n_s dE_s \quad (3a)$$

where  $n_s$  is the number of electrons accepted at the source potential  $E_s$  in a small time interval during which a total of  $n_1$  electrons are accepted. It may be presumed that the relation of  $n_s$  to  $E_s$  is of the nature of a Maxwell-Boltzmann distribution function. The integral is applied over all cytochrome enzymes that accept electrons.

Similar definitions apply to the mean terminal potential,  $\bar{E}_t$ , which is averaged for electrons being accepted by oxygen or other terminal acceptors. Normally the average is to be taken over all electrons leaving the system via cytochrome oxidase. Thus

$$n_2 \bar{E}_t = \int n_t dE_t \quad (3b)$$

where  $n_t$  is the number of electrons donated at the terminal potential  $E_t$  by ferro-cytochrome oxidase to an acceptor during a short time interval in which  $n_2$  electrons are transferred to the acceptor.  $E_t$  is assumed to vary from point to point over the terminal cytochrome and from instant to instant. The integration is over all electrons leaving the system.

The mean difference of cytochrome potential is accordingly given by

$$\bar{E}_{st} = \bar{E}_t - \bar{E}_s \quad (4)$$

and is determined by the mean potentials at which electrons enter and leave the system. Normally it depends, for example, on the ratio of electrons accepted by cytochromes b and c, and on the average ratio of ferri- to ferro-cytochrome oxidase.

In the experiments on the synovial membrane, it has appeared permissible to assume in most cases that the inhibitors and activators affect metabolism only on one side of the membrane, the joint cavity side where the solutions are introduced.

*b* and *c*, and cytochrome oxidase. Each of the components is represented in its standard state (half oxidized, half reduced), in an oxidized state (90%), and in reduced state (90%). The displacement of each of these is 60 mv. from the standard, positive for the oxidized level, and negative for the reduced. This highly simplified schema permits one to visualize roughly the effects of metabolic inhibitors or activators. The diagram represents 3 substances with entirely different effects, each compared with the normal difference in energy level. The inhibitory effect of cyanide on cellular respiration has been explained as resulting from its combination as an iron complex with cytochrome oxidase (4). The cyanide-enzyme complex is no longer able to transfer electrons from the other cytochromes to oxygen. Accordingly, on the energy level diagram, an arrow from the cytochrome *b* level to the cytochrome *c*

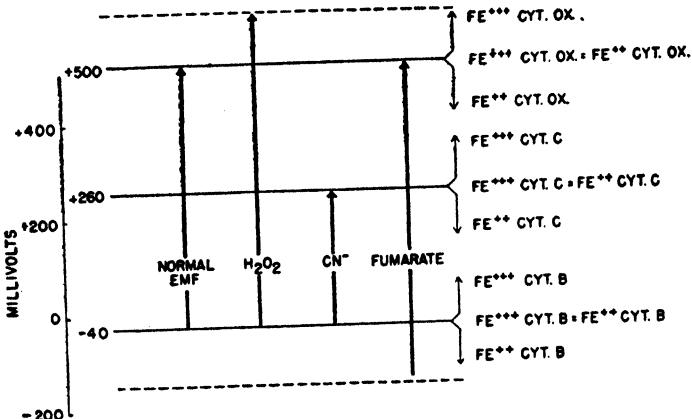


Fig. 2. ENERGY LEVELS IN THE CYTOCHROME SYSTEM, based on values for the standard potentials of the cytochrome components. The length of each arrow is proportional to  $\bar{E}_{st}$ , the mean difference of potential between source and terminal. The values illustrated are for cyanide, hydrogen peroxide, sodium fumarate and the normal untreated tissue.

level represents the mean potential difference,  $\bar{E}_{st}$ , which amounts to 300 mv., compared with the normal difference of 540. According to equation 5,  $E_n = 540 - 300$ , or 240 mv., a value which is uncertain because of the uncertainty of  $E^0$  for cytochrome oxidase. The observed average (table 1) is 180 mv. The calculated value, however, should be approached only for an ideal boundary without transference. The actual system more probably corresponds to a boundary with partial transference. Similar considerations apply to all other cases.

The other examples shown on the energy level diagram (fig. 2) are explained as follows. Hydrogen peroxide, as an electron acceptor or as a source of molecular oxygen formed by the action of catalase, oxidizes cytochrome oxidase, increases the mean terminal potential,  $\bar{E}_t$ , and also the value of  $\bar{E}_{st}$ . According to equation 5 this leads to a negative value of the membrane potential, in contrast with the effects of cytochrome oxidase inhibitors. Fumarate ions stimulate the rate of aerobic metabolism (16, 17). According to Szent-Györgyi, the effect occurs at the succinic dehydrogenase level, where electrons are transferred to ferri-cytochrome *b*, and hydrogen

ions are formed from the substrate. On the energy level diagram, the effect can be represented by indicating the source potential as that of partially reduced cytochrome *b*, the terminal potential remaining at or near its normal level. The use of the diagram in such cases is qualitative and descriptive only as a first approximation. Quite probably in any actual process both source and terminal potentials vary.

TABLE I. CYTOCHROME OXIDASE INHIBITORS AND METHYLENE BLUE

INHIBITOR	NO. EXPER.	CONC. <sup>1</sup>	$E - E_{\text{NaCl}}$	CHARACTERISTICS
Na <sub>2</sub> S	8	0.015	+350	Maximal initially. Drifts negative. Becomes negative after replacement with isotonic NaCl.
Na <sub>2</sub> S + leuco methylene blue (0.01%)	6	0.015	+350	Stabilized by dye.
Na <sub>2</sub> S + oxidized methylene blue (0.1%)	6	0.015 <sup>2</sup>	+270	Stabilized by dye.
NaCN	6	0.03	+180	Stable maximum after short exposure. Reversible with NaCl (2-3 washings).
NaCN + oxidized methylene blue	4	0.03	+180	
Na Azide	4	0.03	+60	Reversible with NaCl.
Hydroxylamine hydrochloride	4	0.03	+80	" " "
Methylene blue (0.01%)	6		+6	" " "

<sup>1</sup> Minimum concentration required to develop given potential. All solutions made isotonic with NaCl.

<sup>2</sup> Solution prepared by aerating leuco dye + Na<sub>2</sub>S.

#### INTERPRETATION OF RESULTS

In the following paragraphs, the experimental results of tables 1 to 5 are discussed with reference to the known effects of the substances as inhibitors or activators. As a basis of discussion, the variations of potential will be represented by equation 5 and by the energy level diagram of figure 2. The observed potentials should correspond to those represented in the figure only in the case of boundaries without transference. All other potentials require correction due to ionic conductance across the membrane.

*Cytochrome Oxidase Inhibitors (Table 1).* A comparison of the effects of sodium cyanide and sodium sulphide at various concentrations is shown in figure 3. The effects of methylene blue on both inhibitors are also shown. The results in table 1 include also the effects of azide and hydroxylamine. All these inhibitors have been recognized to be specific for cytochrome oxidase, preventing the passage of electrons from the other cytochrome components to oxygen (4, 12). It is important to observe with these inhibitors that the maximal effects on the potential occur at higher concentrations (0.015-0.03 M) than are necessary to cause practically complete inhibition of

cellular respiration ( $0.001\text{ M}$ ). At these lower concentrations the membrane potential with cyanide or sulphide is of the order of 20 or 30 mv., compared with the maximal of 150 to 400 mv. developed at higher concentrations. There is, accordingly, no direct proportionality between the inhibition of oxygen consumption and the membrane potential produced by one of these substances. Therefore,  $\bar{E}_{st}$  does not necessarily indicate the rate of flow of electrons over the cytochromes. This rate corresponds to  $n_2$  of equation 3b, which is the number of electrons yielded to oxygen per unit of time. As  $n_2$  approaches zero for complete inhibition at low concentration,  $E_m$  shows no corresponding approach to a maximum (fig. 3). This fact can be expressed by the statement that the ratio  $\bar{E}_{st}/n_2$  becomes maximal in the presence of the inhibitor. As this is the ratio of a potential difference to a current, it can be taken to express resistance in the cytochrome system. Thus the effect of the inhibitor can be represented.

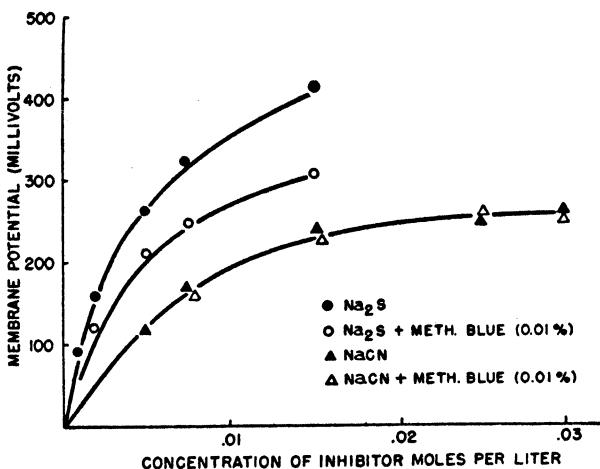


Fig. 3. RELATION BETWEEN MEMBRANE POTENTIAL and concentration of inhibitor for cyanide and sulphide inhibition. Results given both in presence of methylene blue and in its absence. All solutions made isotonic with  $\text{NaCl}$ .

sented as a large increase of this resistance. For complete inhibition of tissue respiration,  $\bar{E}_{st}$  is essentially a static difference of potential; for normal tissue respiration it is a difference of potential maintained with a constant electron current, as long as the metabolic rate is constant.

To describe, by means of the energy level diagram, the effect of  $\text{NaCN}$ , the terminal potential  $\bar{E}_t$  has been represented as dropping from its assumed normal level of 500 mv. to the cytochrome *c* level, 260 mv. Ball has favored the hypothesis that cytochrome oxidase reacts with its inhibitors to form complexes with lower standard potentials than that of the free enzyme (12). The potential of each complex is specific. For example that of the azide complex is considered higher than that of the cyanide complex. The experimental results on membrane potentials can be interpreted from this point of view, the maximum potential obtained with cyanide being found roughly to correspond to a terminal potential at the cytochrome *c* level. As

previously explained, allowance must be made for the effects of transference on the observed potentials.

Typically, the effect of sulphide on the membrane potential is considerably greater than that of cyanide. In the presence of oxidized methylene blue, the potential of sulphide tends to approach that of cyanide at the same concentration. Methylene blue has little or no effect on the cyanide potential curve (fig. 3). The effects can be interpreted in the following way. In the presence of sulphide,  $\bar{E}_t$  corresponds to a level for greatly reduced cytochrome *c*. Oxidized methylene blue tends to re-oxidize the reduced cytochrome *c* to a level nearer that occurring in cyanide inhibition. An enzyme, cytochrome peroxidase, which catalyzes the oxidation of cytochrome *c* by hydrogen peroxide has been described (18). Methylene blue, as an intermediary carrier from metabolites to oxygen, forms hydrogen peroxide. This auxiliary pathway does not appreciably change the terminal potential in the case of cyanide inhibition, presumably because the cytochrome *c* is at a more oxidized level. The cyto-

TABLE 2. EFFECTS OF THIOLS

THIOL	NO. EXPER.	CONC. <sup>1</sup>	$E - E_{\text{NaCl}}$	CHARACTERISTICS
		mol./l.	at. in mv.	
Thiourea	8	0.03	+220	Maximum after 3-5 min. Reversible with NaCl.
Urea	4	0.03	+4	Reversible
Thiouracil	6	0.01 <sup>2</sup>	+50	Maximum after 3-5 min. Reversible.
Glutathione	5	0.01	-10	Reversible.
Sodium thioglycolate	8	0.03	+170	Maximum after 3-5 min. Reversible.

<sup>1</sup> Minimum concentration required to develop given potential. All solutions made isotonic with NaCl.

<sup>2</sup> Limited by solubility.

chrome peroxidase system is a possible mechanism for maintaining the cytochrome *c* potential level, but one which is not sufficient to compete with the strong reducing action of the hydrosulphide ion. Wieland and Sutter (19) have demonstrated the inactivation of peroxidase by sulphide. Thiols, such as thioglycolic acid, produce high positive membrane potentials, which also appear to be explicable as reducing action at the cytochrome *c* level.

*Effects of Thiols (Table 2).* With the exception of glutathione, none of the thiols included in table 2 is a natural constituent of biological fluids. Urea is included in the table for comparison with thiourea. Thioglycolate, thiourea and thiouracil all show high positive potentials. All are metabolic inhibitors. Glutathione, however, shows a small negative potential. The effects of the thiol inhibitors can be interpreted as the transport of electrons to the cytochrome *c* level rather than to cytochrome *b*. The inhibitors thus compete with normally active enzymes and carriers, carrying the electrons to a higher source potential.  $\bar{E}_s$  then tends to approach the cytochrome *c* potential, which is approximately 300 mv. higher than that of cytochrome *b*. Observed potentials with thiourea and thioglycolate are often greater

than 200 mv. Thiouracil shows smaller effects, but is only slightly soluble ( $0.01\text{ M}$ ). Partial transference again must be considered to affect the results. As the first approximation, however, the results can be expressed by means of *equation 5* or the energy level diagram where the source potential,  $\bar{E}_s$ , is taken as more positive than normal. This type of inhibition differs from the cyanide type in which the terminal potential is taken as less than normal. Both effects result in high positive observed potentials.

*Heavy Metals and Other Sulfhydryl Inhibitors (Table 3).* Heavy metals influence metabolism in numerous ways. They combine with the sulfhydryl groups both of enzymes and of thiols such as glutathione (20,21). They are very active catalysts for oxidoreductions involving thiols, and accordingly affect the pathways of the respiratory systems. In addition, they are involved in other types of complex systems,

TABLE 3. HEAVY METALS AND OTHER SULPHYDRYL INHIBITORS

INHIBITOR	NO. EXPER.	CONC. <sup>1</sup>	$E - E_{\text{NaCl}}$	CHARACTERISTICS	
				mol./l.	av. in mv.
CuCl <sub>2</sub>	12	0.00075	+180		Stable for several min. Difficult to reverse with NaCl. Reversible with 0.4% glutathione in NaCl.
FeCl <sub>3</sub>	8	0.0075	+220		Initial maxima which drift toward negative. Reversible with several washings in NaCl. Easily reversed with 0.015 M Na pyrophosphate.
HgCl <sub>2</sub>	6	0.00075	+180		Difficultly reversed. Reversible with glutathione (0.4%).
p chlormercuribenzoate	6	0.003	+50		Maximum in 4-5 min. Reversible with NaCl.
Na o-iodibenzoate	6	0.00075	+100		Maximum in 4-5 min. Reversible with NaCl.
Na iodoacetate	5	0.015	+100		Develops rapidly. Reversible with NaCl.

<sup>1</sup> Minimum concentration necessary to develop given potential. All solutions made isotonic with NaCl.

such as the iron enzymes, catalase, peroxidase and the cytochromes, as well as the copper containing protein complexes, hemocuprein and hepatocuprein (22). Further indications of the complexity of their behavior are given by the results of Ames, Ziegenhagen and Elvehjem (23) who have found that various inhibitors are specific for iron and copper, as well as for various respiratory enzymes. Substances that inhibit iron retard the oxidation of ascorbic acid, copper inhibitors impede the oxidation of glutathione, while the oxidation of succinate depends on both. Cytochrome *c* catalyzes the oxidation of these substrates, the oxidation of succinate requiring the enzyme succinic dehydrogenase (4). These facts are in agreement with the effects of ferric and cupric ions on the membrane potentials, which are of the order of 200 mv. at the most effective concentrations. The metals, by catalyzing the oxidation of one or more of the systems, glutathione, ascorbic acid, succinic acid, establish an auxiliary pathway by which electrons are separated from protons at

the cytochrome *c* level, rather than at cytochrome *b*.  $\bar{E}_s$  is thus increased, and  $\bar{E}_{st}$  decreased. This effect would be sufficient to explain the result, and the similarity with the effects of the thiol inhibitors. Actually, however, the metals probably react in several additional ways. According to Keilin and Hartree (24), copper oxidizes all the cytochrome components. Also, the heavy metals act as sulfhydryl inhibitors, decreasing the rate of glycolysis and other enzymatic dehydrogenations at potentials below the cytochrome *b* level.

The sulfhydryl inhibitors, p-chloromercuribenzoate, o-iodobenzoate and iodoacetate act more specifically on the latter processes (25). All of them produce positive membrane potentials, which may be interpreted as caused by an increased value of the mean source potential,  $\bar{E}_s$ . This would be a direct result of slowing the rate at which electrons are transferred to cytochrome *b*.

*Succinic Dehydrogenase Inhibitors, Activators and Substrate (Table 4).* Malonate is an inhibitor which acts with some specificity on the enzyme succinic dehydrogenase (17, 26). When introduced into the joint cavity, it produces a positive potential of about 50 mv., which, however, soon drifts toward zero. The positive potential is easily explained as the result of an increased potential due to inhibition of electron transport through succinic dehydrogenase. The drift could possibly be explained as a subsequent oxidation of the terminal cytochrome oxidase with an increase of  $\bar{E}_t$ . Again it is evident that there is no necessary direct connection between the metabolic rate and the membrane potential. With strong inhibition,  $\bar{E}_{st}$  is essentially a static difference of potential, and may have a value very close to normal.

Sodium fumarate stimulates metabolism through the succinic dehydrogenase system (16, 17). It produces a negative membrane potential of about 30 millivolts (table 4). This persists after numerous washings with NaCl, indicating that the fumarate is firmly bound to the enzyme. In this case, the source potential is more negative than normally because of the greater rate at which cytochrome *b* is reduced by the substrate-enzyme system. The source potential,  $\bar{E}_s$ , evidently depends on the rates at which electrons are added to and removed from cytochrome *b*. It represents essentially a balance between the rates of oxidoreductions in the cytochromes and in the dehydrogenation processes which supply electrons to cytochrome *b*. Sodium succinate yields a negative potential of the order of 20 mv., which drifts toward zero. It is ineffective at low concentrations, and seems to behave simply as a substrate rather than as a coenzyme.

*Electron Acceptors (Table 5).* Iodine and hydrogen peroxide are placed in this group because their effects on the potential appear to indicate that initially at least they oxidize at the cytochrome oxidase level. The instability of the initial negative potentials, which drift toward positive, may indicate a slower tendency to oxidize the other cytochrome components. The behavior of iodine differs in solutions containing iodide ions compared with those that contain only chloride ions. In the latter solutions the initial potentials are of the order of minus 50 mv., but they rapidly drift beyond zero and become quite positive. In solution with iodide ions, iodine always yields positive potentials. The value depends on the concentration of the iodide ions. In the presence of 0.15 M NaI, the potential given by 0.0015 M iodine

is approximately 200 mv. At lower concentrations of iodide ion, the solutions being made isotonic with NaCl, the potentials are lower, but continuously drift positive. Isotonic NaI itself yields a positive potential of about 25 mv. (1).

The above facts suggest that iodide and iodine act in an auxiliary hydrogen transport system which is capable of carrying electrons to the cytochrome *c* level. The initial effect of iodine appears to be that of an electron acceptor at the cytochrome oxidase level, producing increases of  $\bar{E}_t$  and  $\bar{E}_{st}$ . As soon as traces of iodide ion are formed the carrier system operates, and the secondary effect is to increase  $\bar{E}_s$ . Accordingly,  $\bar{E}_{st}$  decreases, and the observed potential becomes positive. Since molecular iodine is capable of oxidizing glutathione, this may possibly be part

TABLE 4. INHIBITORS AND ACTIVATORS OF SUCCINIC DEHYDROGENASE

REAGENT	NO. EXPER.	CONC. <sup>1</sup>	$E - E_{NaCl}$	CHARACTERISTICS	
				mol./l.	av. in mv.
Na malonate	8	0.01	+50		Unstable initial maximum. Drifts toward zero.
Na fumarate	8	0.015	-30		Immediate effect which persists after several washings with NaCl.
Na succinate	6	0.075	-20		Unstable initial potential. Drifts toward zero.

<sup>1</sup> All solutions made isotonic with NaCl.

TABLE 5. ELECTRON ACCEPTORS

REAGENT	NO. EXPER.	CONC.	$E - E_{NaCl}$	CHARACTERISTICS	
				mol./l.	av. in mv.
I <sub>2</sub> in 0.15 M NaCl	12	0.0015	-50		Initial negative potential which rapidly drifts positive.
I <sub>2</sub> in 0.15 M NaI	12	0.0015	+200		Partially reversed with NaCl. Potential maintained after washing with isotonic NaI.
H <sub>2</sub> O <sub>2</sub> in 0.15 M NaCl	8	0.01	-70		Drifts positive slowly. Reversible with NaCl.

of the system involved. The mechanism might also account for the potentials observed with isotonic iodide, which are abnormally positive compared with those of chloride and bromide. Tissues oxidize iodide ion by means of peroxidase (27). Thus the carrier system responsible for the positive potential would be formed either from the ion or from molecular iodine. This mechanism results in inhibition similar to that given by the heavy metal-thiol systems, which also yield positive potentials. All are explicable as oxidations of cytochrome *b* by transport of electrons and protons to higher cytochrome levels through the action of auxiliary hydrogen transport systems.

Hydrogen peroxide produces an initial negative potential, which ultimately drifts toward zero. Its initial effect is to increase  $\bar{E}_{st}$  by oxidizing cytochrome oxidase (fig. 2). The secondary positive drift is possibly the subsequent effect of ox-

dations of the other cytochrome components, possibly through cytochrome peroxidase (18).

*Relation to Polarization Potentials.* An earlier investigation of the electrochemical properties of the synovialis included experiments on the potentials produced by polarization of the membrane brought about by application of an external electro-motive force (1). For voltages above a certain threshold applied for two or more minutes, the polarization potentials that were produced were often noted to be fairly stable for as long as 5 minutes, depending on the polarizing current and the time it had been applied. The level of the stable polarization potential was nearly always observed to be between 150 and 200 mv. The sign was determined by the direction of the applied current, either positive or negative. The magnitude was as a first approximation independent of the applied voltage as long as this exceeded the threshold level. Reconsideration of these facts suggests that they are explicable in terms of the mechanism proposed in the theoretical section of this paper. An externally applied field necessarily induces a displacement current of electrons in the cytochrome system as well as migration of ions. Electrons would be displaced toward the anode, reducing ferri-cytochrome oxidase to the ferro-enzyme on the anodic side of the membrane. Eventually, the terminal cytochrome potential would be reduced to the cytochrome *c* level, where presumably it stabilizes because the concentration of the *c* component is much greater than that of the others. The galvanically induced reduction of cytochrome oxidase that is postulated leads to inhibition on the anodic side of the membrane. The type of inhibition is that in which the mean terminal potential,  $\bar{E}_t$ , decreases, dropping toward the cytochrome *c* level. With removal of the applied current, this displaced potential would manifest itself as a polarization potential, which persists as long as the cytochrome oxidase is reduced. Thus the length of time during which the polarization potential is stable depends on the quantity of cytochrome *c* reduced during the period of polarization. This agrees with the experimental evidence which shows that it is the time of stability rather than the potential level which is proportional to the quantity of current transferred. The magnitude of the potential, 150 to 200 mv., also is in agreement with the postulate, for it agrees fairly well with that observed with cyanide as an inhibitor, in which case the terminal potential is regarded as dropping to the cytochrome *c* level. The above considerations indicate that there is a reciprocal relation between metabolism and potentials; metabolism may depend on induced galvanic currents, and conversely potential gradients in tissues depend on metabolic rates and pathways.

#### DISCUSSION

The relation of polarization potentials, and bioelectric fields in general, to growth and metabolism have been discussed in a recent monograph by Lund (28). As experimental material, vegetable preparations were most extensively studied. The results show numerous interrelations between the bioelectric fields, polarization in applied fields and metabolism as influenced by inhibitors. The magnitudes of the potentials quite clearly are beyond those predicted for ionic diffusion potentials. The relation of membrane potentials to metabolism has been a controversial sub-

ject (29). Although earlier work has shown that metabolic inhibitors produce high potentials, the origin of the potential has been a source of disagreement. A magnitude of the order of 100 mv. would require an ionic concentration gradient of about 100 to 1 across a membrane with very high selectivity toward one of the ions. Under the conditions of these experiments, no such ratio could possibly develop. The earlier results on metabolically inactive ions indicate very little selectivity by the synovial membrane (1).

In accounting for the potentials as originating in tissue oxidoreductions the difficulty consisted in accounting for an electron transfer mechanism (29).

In boundaries with transference, high potentials resulting from oxidoreductions are theoretically impossible. The cytochrome system, conceived as an electron conductor, resolves this difficulty by providing a natural boundary with partial transference. The system can be conceived as oriented toward the oxygen supply of the tissues, with the *b* and *c* components taking up electrons at a low potential and conducting them rapidly to the oxidase at a high potential (5). The normal potential difference in the system is of the order of 500 mv. Because of symmetrical orientation, the normal membrane potential is the resultant of two nearly equal and opposite vectors. It therefore is of a small order of magnitude. Inhibitors or activators can selectively act on one of the component vectors, and produce high potentials. Polarization in an external field can lead to a similar result. Both effects are readily accounted for by the assumed mechanism.

#### SUMMARY

The effects of various types of inhibitors and activators on the potential of the synovialis in dogs have been determined. Among the agents studied were cytochrome inhibitors, sulphydryl inhibitors, thiols and inhibitors of succinic dehydrogenase.

Theoretical considerations based on the properties of artificial boundaries with and without transference have been presented to facilitate the interpretation of the results. On the basis of the theoretical considerations, the observed potentials have been related to the known metabolic effects of the inhibitors and activators. The effects are referred to mean differences of potential between the source and terminal in the cytochrome system. Inhibitors, in general, by decreasing this difference of potential on one side of the membrane, produce a high positive potential across the membrane. Activators show the opposite effect. The magnitude and sign of the potential can be related to the effects on enzymes, coenzymes or carriers. The results are discussed in connection with an energy level diagram giving the standard potentials of the cytochrome components.

It is shown that a galvanic current can lead to inhibition on the anodic side of the membrane, and that the relation between metabolism and the electric field is reciprocal.

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# CORRELATION BETWEEN SIGNS OF TOXICITY AND CHOLINESTERASE LEVEL OF BRAIN AND BLOOD DURING RECOVERY FROM DI-ISOPROPYL FLUOROPHOSPHATE (DFP) POISONING

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SEVERAL investigators have reported that for the brief period immediately following the injection of di-isopropyl fluorophosphate (1, 2), a close relationship exists between the severity of toxic signs and the degree of depression of brain cholinesterase activity, a relationship which with one exception (3), has not been considered coincidental. However, during the more prolonged recovery from DFP the relationship between toxic signs and brain cholinesterase activity has not been explored. Yet studies of Grob, *et al.* (4) on man indicate the necessity of such data in explaining the behavioral changes. These investigators found that following recovery from a large dose of DFP, clinical signs of toxicity disappeared within 48 hours while the red blood cell cholinesterase activity remained stationary during that time. Erythrocyte cholinesterase activity has been considered a reflection of brain cholinesterase activity because of the parallelism between the depression of the cholinesterase activity of erythrocytes and that of brain by DFP *in vitro* (3) and *in vivo* in the acute stages of DFP poisoning. One may, therefore, conclude from the experiments of Grob *et al.* (4) that brain cholinesterase remains stationary during the 48-hour period while the toxic signs disappear. In other words, the brain becomes acclimatized to function relatively normally at a level of tissue cholinesterase considerably below the normal of optimal range. Another explanation of this experiment would be that the brain cholinesterase regenerates more rapidly than the erythrocyte cholinesterase. The authors questioned the latter possibility because Mazur and Bodansky (3) and Koelle and Gilman (1) had reported that in the rabbit and rat the red blood cell cholinesterase returns to normal before the brain cholinesterase activity. Nevertheless the data of Koelle and Gilman (1) on the rat suggests that during a limited period immediately following the injection of DFP the rate of regeneration of erythrocyte cholinesterase is slower than brain cholinesterase. In order to investigate more completely the rates of regeneration of the cholinesterase of brain and blood and, in that way, study the relationship between cholinesterase activity and toxic signs the following experiments were undertaken.

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Received for publication December 7, 1948.

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#### METHOD

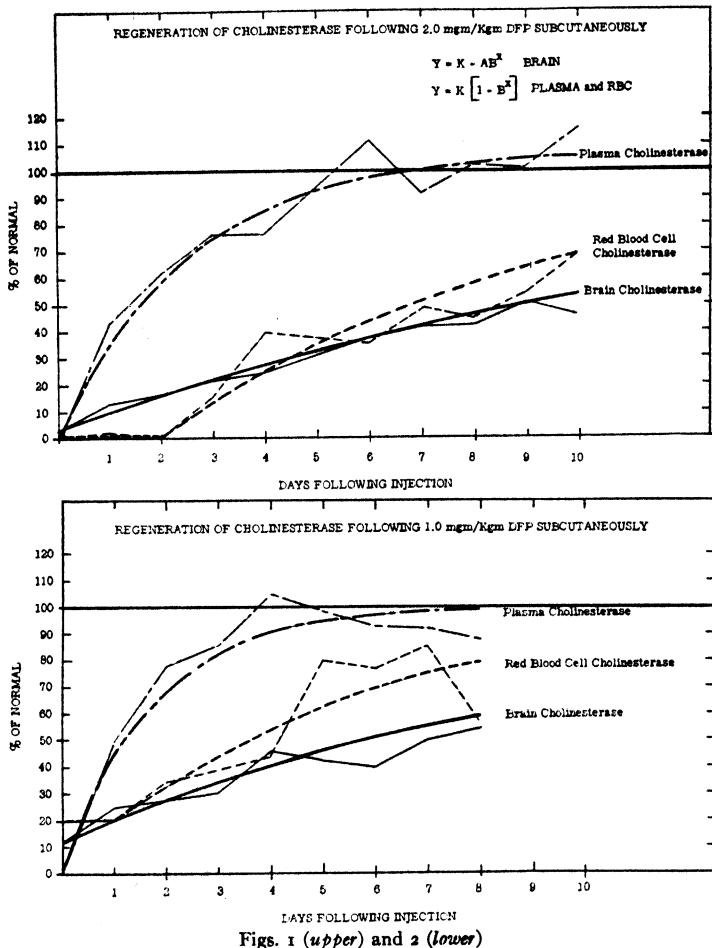
A total of 94 adult male rats weighing between 125 and 175 grams were used. The animals were divided into groups of 20 to 25 animals and each group was studied in the following manner. All rats were injected subcutaneously either with 1.0 or 2.0 mg/kg. DFP in saline, every member of a given group receiving the same dose. One hour following the injection, the rats in the group were observed for toxic signs. Subsequently, the animals were inspected daily. The manifest signs of toxicity at each inspection time appeared quite uniform within each group. Toxic death occurred chiefly during the first hour when approximately 25 per cent of the rats died with the 2.0-mg. dose and less than 10 per cent succumbed with the 1.0-mg. dose. Only animals with longer survival periods were examined for behavioral and biochemical changes.

At each observation two rats were selected at random from the group. Each of these rats was carefully studied noting all behavioral signs of toxicity. In evaluating the signs of toxicity, the following criteria were used: Animals were graded 'severe' where they displayed evidences of grave toxicity such as marked spontaneous trembling of the body, great hyperreactivity upon tapping the spine, muscular weakness of varying degrees culminating in paryses, and excessive salivation. Rats graded as of 'moderate' toxicity exhibited manifestations including intermittent periods of trembling, marked motor restlessness, moderate hyperreactivity upon tapping the spine and spontaneous fasciculations of the muscles in the flanks. Animals rated as exhibiting 'slight' toxicity showed signs such as transient fasciculations while standing erect on their hind legs and motor restlessness. Animals that were free of overt toxic signs were considered 'normal'. Members of each group were studied until the remaining members became free of toxic signs. Then these rats were killed and the cholinesterase activity of the whole brain, packed erythrocytes and plasma were determined. The brain cholinesterase was determined as described in previous work (2). The erythrocytes and plasma cholinesterase were also measured manometrically. The erythrocytes were not washed but carefully separated from the plasma after prolonged centrifugation in a hematocrit tube. It was found that better agreement between samples obtained from control rats could be found without washing although the mean activity was not significantly altered. A further correction was necessary in determining the cholinesterase activity of the erythrocytes. Because their anaerobic glycolysis produced significant amounts of CO<sub>2</sub>, a sample without acetylcholine was run simultaneously with each erythrocyte determination and the figure so obtained subsequently subtracted. Control values for the brain and blood cholinesterase activity were obtained from 17 normal rats.

#### RESULTS

Figure 1 indicates regeneration of cholinesterase in the brain, plasma and erythrocytes following a dose of 2.0 mg/kg. of DFP. In this and subsequent charts the broken line is drawn through the determined points. It was found that these data could be best described mathematically in all cases by the equation Y = K - AB<sup>x</sup>

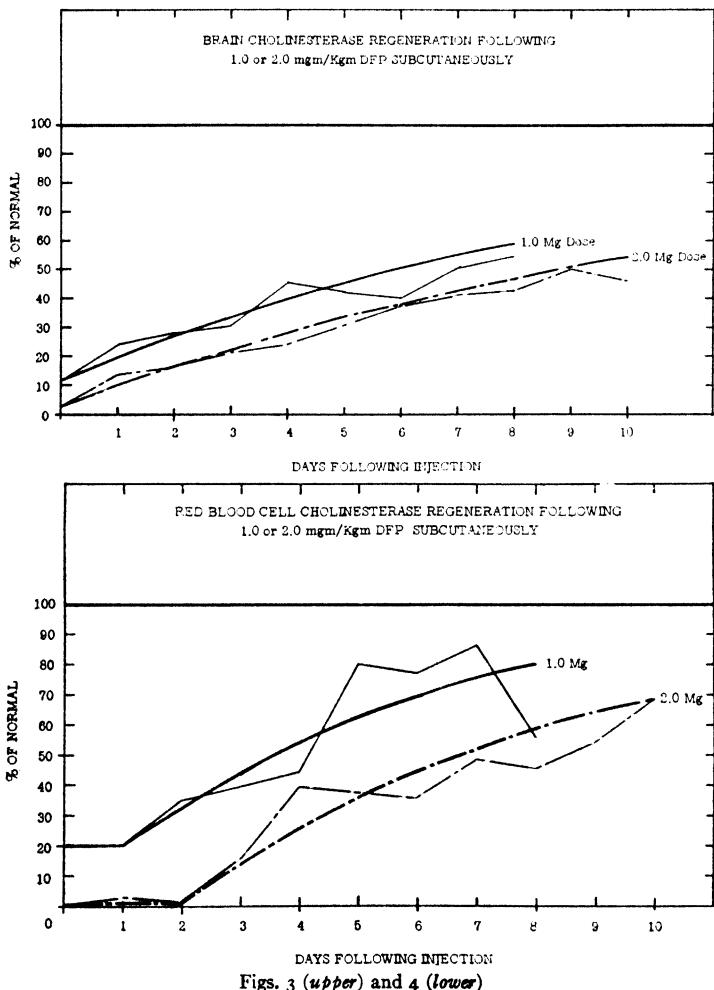
for the brain and  $Y = K(1 - B^x)$  for the plasma and erythrocytes. These equations are represented by the smooth curves that describe the regeneration plasma, red blood cell, and brain cholinesterase respectively. The nonspecific plasma cholinesterase regenerated most rapidly and reached normal within 7 days. On the other



Figs. 1 (upper) and 2 (lower)

hand during the first 48 hours the specific erythrocyte cholinesterase activity remained almost at zero while the brain cholinesterase increased more than threefold. Subsequently the rate of red blood cell cholinesterase increased and the red blood cell line in the figure can be seen crossing the brain line. A similar study was performed with rats injected with 1.0 mg/kg. DFP as indicated in figure 2. Again a lag in the regeneration of red blood cell cholinesterase activity is observed. In this instance

the lag was only 24 hours during which time the brain cholinesterase was almost doubled. The similarity in the regeneration patterns when either 1.0 or 2.0 mg/kg. DFP was injected is further emphasized in figure 3 where the brain cholinesterase for each dose is plotted. The parallel in the trend lines is apparent. Similarly in



Figs. 3 (upper) and 4 (lower)

figure 4 the erythrocytes cholinesterase activity for both the 1.0 and 2.0 mg/kg.-dose are indicated. In each case one observed first a lag and then parallel lines of regeneration.

Table 1 indicates the relationship between the severity of the clinical signs and the levels of the various cholinesterase activities in the rats injected with 2.0 mg/kg.

The mean time of appearance in days of each of these symptom groups is likewise indicated. One notices the steady increase in the means of the brain cholinesterase activity and the improvement in clinical signs.

The scatter of the brain cholinesterase corresponding to each clinical category is not wide as can be seen from the low standard error of the mean. Furthermore, by the *t*-test it has been found that each of the mean brain cholinesterase activities is significantly different from every other (table 2). This indicates that the classification is valid and that each category is sharply delineated from the next. In the case

TABLE I. RELATIONSHIP OF SIGNS OF TOXICITY TO CHOLINESTERASE ACTIVITY FOLLOWING 2.0 MG/KG.

SYMPTOMS	BRAIN CHOLINESTERASE % NORMAL	RBC CHOLINESTERASE % NORMAL	PLASMA CHOLINESTERASE % NORMAL	APPEAR- ANCE, TIME-DAYS
Severe.....	2.9 ± .3 <sup>1</sup>	○	○	.042
Moderate.....	17.9 ± 2.0	10.4 ± 3.9	59.8 ± 10.8	2.35
Slight.....	35.9 ± 2.5	37.5 ± 6.7	93.3 ± 14.7	5.78
None.....	46.0 ± 2.5	58.3 ± 8.1	111.3 ± 16.0	8.80
Control Values.....	1081.2 ± 14.1 mm <sup>3</sup> CO <sub>2</sub> /hr/100 mg. Brain	1175.2 ± 41.6 mm <sup>3</sup> CO <sub>2</sub> /hr/1 cc. packed RBC	511.3 ± 29.4 mm <sup>3</sup> CO <sub>2</sub> /1 hr/1 cc. plasma	

<sup>1</sup> Standard error on % of mean.

TABLE 2. SIGNIFICANCE OF DIFFERENCE BETWEEN MEANS OF CHOLINESTERASE VALUES CORRESPONDING TO CATEGORIES OF TOXIC SIGNS FOLLOWING 2.0 MG/KG DFP

*p* Values determined in *t* test

SOURCE OF CHOLINESTERASE	SEVERE AND MODERATE	MODERATE AND SLIGHT	SLIGHT AND NONE
Brain.....	<.01 sig.	<.01 sig.	<.01 sig.
RBC.....	.02 ? sig.	<.01 sig.	.035 ? sig.
Plasma.....	<.01 sig.	.025 ? sig.	.24 not sig.

A value less than .01 means that the difference in the means is significant since this difference could only occur 1 time out of 100 by chance. Values from .01 to .05 are of questionable significance. Values greater than .05 indicate that the two means in question are not significantly different.

of the RBC cholinesterase values the relationship with toxic manifestations is not as clear-cut. The scatter is greater and each category is not as sharply demarcated from the next (table 2). The plasma cholinesterase values are even less consistent. The scatter is greater and the categories are not as well differentiated.

In a similar fashion table 3 indicates the relationship between the severity of clinical signs and the levels of the various cholinesterases in the rats injected with 1.0 mg/kg. Again the most consistent relationship is between the brain cholinesterase and toxic signs. Each clinical category is sharply demarcated and significantly different from the next as determined by the *t* test (table 4). As with the

larger dose, the relationship between clinical signs of toxicity and RBC cholinesterase is less consistent and plasma cholinesterase still less (tables 3 and 4).

It appeared of interest to compare the mean brain cholinesterase corresponding to categories of clinical signs following a 1.0 mg. and 2.0 mg./kg. dose with each other. This has been done in table 5. Furthermore, similar determinations previously made in the acute state (30 minutes following the injection of DFP) are also listed. A con-

TABLE 3. RELATIONSHIP OF SIGNS OF TOXICITY TO CHOLINESTERASE ACTIVITY FOLLOWING 1.0 MG./KG. DFP

SIGNS	BRAIN CHOL. % NORMAL	RBC CHOL. % NORMAL	PLASMA CHOL. % NORMAL	APPEARANCE OF SIGNS—MEAN TIME IN DAYS
Moderate.....	12.0 ± 1.1	19.5 ± 4.5	○	.042
Slight.....	31.6 ± 2.3	39.5 ± 7.1	77.8 ± 11.6	2.70
None.....	52.4 ± 2.9	68.4 ± 10.2	90.5 ± 11.8	7.43

Control values as in table 1.

TABLE 4. SIGNIFICANCE OF DIFFERENCE OF MEANS CORRESPONDING TO CATEGORIES OF TOXIC SIGNS FOLLOWING 1.0 MG./KG. DFP

*p* Values determined in *t*-test

	MODERATE AND SLIGHT	SLIGHT AND NONE
Brain.....	<.01 sig.	<.01 sig.
RBC.....	.05 ? sig.	.02 ? sig.
Plasma.....	<.01 sig.	.28 not sig.

See table 2.

TABLE 5. CORRESPONDENCE OF MEAN BRAIN CHOLINESTERASE PER CENT NORMAL AND CATEGORIES OF CLINICAL SIGNS UNDER VARYING CONDITIONS

CLINICAL CATEGORIES	PERIOD OF REGEN. FOL- LOWING 1 MG./KG. DFP	PERIOD OF REGEN. FOL- LOWING 2 MG./KG. DFP	30 MIN. FOLLOWING VARIOUS DOSES DFP <sup>1</sup>
			%
Moderate.....	12.0	17.9	11
Slight.....	31.6	35.9	33
None.....	52.4	46.9	

<sup>1</sup> From Freedman and Himwich (2)

sistency is observed in the level of the brain cholinesterase activity for each of the three states with the possible exception of those values corresponding to moderate signs.

Since in the acute state the RBC cholinesterase seemed to parallel the brain cholinesterase closely, it was of interest to note if any such correlation existed in the overall regenerative period studied in these experiments. The correlation coefficient of RBC with brain cholinesterase following a 1.0 mg./kg. dose of DFP was determined and found to be .74. Thus, one could use RBC cholinesterase to approximate

changes in the brain during regeneration as performed here. This would be more accurate than determining plasma cholinesterase both in the acute or recovery phase.

#### DISCUSSION

It appears from these data that there is a reversal of rates of regeneration between red blood cell cholinesterase and brain cholinesterase. In spite of the fact that erythrocytes cholinesterase returns to normal sooner than the brain, yet during the earliest period the brain regenerates more rapidly than the red blood cells. Thus the problem posed by Grob *et al.* (4) concerned with the early differences of the clinical signs may be explained by the early lag in erythrocyte regeneration in contrast with the simultaneous rapid regeneration of brain cholinesterase.

Our rats though exhibiting great powers of recovery nevertheless retained signs of toxicity longer than the human subjects. Needless to say such low levels of cholinesterase activity as reached in the rat were not obtained in man. Furthermore, these data indicating rapid destruction of cholinesterase by DFP and its relatively slower regeneration point out the potentialities of this drug for accumulation on repeated administration.

Additional correlative evidence is furnished in this work that toxic signs in DFP poisoning closely parallel changes in brain cholinesterase activity during recovery from a dose of DFP. It is possible that some of the toxic signs observed are not directly related to the central nervous system and it may well be that if one were to determine the regenerative pattern of cholinesterase activity in the neuromyal junction a similar or closer parallelism might be found. The fact remains however that this correlation exists between toxic signs and biochemical changes. It further appears that for each general level of brain cholinesterase activity one can expect approximately the same overt manifestations of toxicity as observed whether the animal is in the acute or recovery phase and this obtains whether a dose of 2.0 or 1.0 mg/kg. is used. It is noteworthy that brain cholinesterase activity can be reduced to about half before the earliest overt sign of toxicity develop. Of course, between the levels of 50 to 100 per cent normal cholinesterase activity changes in brain function may be present that require finer methods than those employed in the present investigation for their revelation. In any case, it should be pointed out a significant excess of cholinesterase appears to be present beyond that which is needed for normal function. All this suggests that brain cholinesterase plays an important role in the normal animal in the maintenance of nervous activity and that changes in brain cholinesterase activity are accompanied by corresponding alterations in the function of the central nervous system.

#### SUMMARY

Following the injection of large doses of DFP nonspecific plasma cholinesterase regenerates more rapidly than the specific cholinesterases of the red blood corpuscles and the brain. A lag in regeneration of erythrocyte cholinesterase activity lasting from 24 to 48 hours occurs following the injection of DFP, a period during which brain cholinesterase regenerates rapidly. Subsequently, the rate of regeneration of erythrocyte cholinesterase becomes more rapid and surpasses that of the brain. A close

relation appears to exist between the severity of the toxic signs of DFP poisoning and the level of brain cholinesterase during the regeneration period. The relation between toxic signs and erythrocyte cholinesterase activity is less exact and fails altogether in the case of the plasma.

We gratefully acknowledge the statistical analyses done by Miss Frieda Faiman.

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# FACTORS IN EXPLOSIVE DECOMPRESSION INJURY<sup>1</sup>

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**C**ONVENTIONAL studies on explosive decompression have involved the sudden alteration of the environment from that of the normal, ambient atmosphere to rather high vacua, in simulation of pressure-cabin failure. In such experiments, therefore, test animals are simultaneously subjected to: a) the rapid expansion of contained gases; b) anoxic anoxia; and c) aeroembolism (at sufficiently reduced pressures). Qualitative evaluation of the relative importance of these factors in the etiology of injury and death from rapid decompression has thus been difficult. Other methods of experimental procedure were therefore adopted in the hope of further elucidating this phase of the problem.

## METHODS

In over 300 individual tests, rats were compressed within 30 seconds to pressures of from 2 to 30 atmospheres, maintained for various time periods (10 seconds to 30 minutes). Subsequent decompression to normal atmospheric pressure was rapid (average time, 0.62 seconds, as determined by high-speed motion pictures). Adequate ventilation of the chamber was maintained throughout, the flow of air being measured by means of a gas meter. In this manner an anoxic environment was eliminated from the conditions of the experiment, and the effects of gas expansion, only, could be observed. It is obviously quite possible that nitrogen narcosis may have been present in animals placed under the higher pressures, but recognizable symptoms of this condition were not detected. It is moreover true that pressure changes in this type of experiment were many times greater than in tests of the conventional sort, but it was hoped to obtain by the method described an evaluation of the effectiveness of intrapulmonary gas expansion as a lethal agent.

The effects of anoxia were observed by placing animals in an atmosphere of nitrogen for suitable intervals. All rats which succumbed were examined for the presence of gross lesions, hemorrhage and the presence of gas emboli. Those surviving were kept under observation for periods of from 1 to 4 weeks, at the end of which time several rats from each experimental group were killed for study. In none of the latter animals, however, was gross evidence of decompression injury present at the time of autopsy.

The results of all experiments involving decompression are given in table I. As shown in figure 1, explosive decompression following 30-minute exposures to 2 to 6 atmospheres (gas-expansion equivalent, 18,000 to 42,000 ft.) was well tolerated by 59

Received for publication January 25, 1949.

<sup>1</sup> Work carried out under a contract with the U. S. Navy, Office of Naval Research, and the University of Virginia.

rats. No gross pathological lesions were later observed in these animals. Following decompression from 7 atmospheres, half of the animals succumbed, without discernible injury, and mortality rates were slightly increased following release from 8 and 9 atmospheres of pressure. Decompression from 10 atmospheres resulted in the death of 14 of 15 rats (93%) and all (20) animals succumbed on rapid release from 11 atmospheres.

TABLE I. EFFECTS OF EXPLOSIVE DECOMPRESSION FROM HIGH BAROMETRIC PRESSURE TO THAT OF AMBIENT ATMOSPHERE

PRESSURE FROM WHICH DECOM- PRESSED	APPROXIMATE GAS EX- PANSION EQUIVALENT ON DECOMPRESSION, EXPRESSED AS ALTITUDE	TIME AT HIGH PRESSURE	NO. OF ANIMALS TESTED	TOTAL DEATHS		ANIMALS SHOW- ING PULMONARY LESIONS		ANIMALS SHOWING AEROEMBOLISM	
				No.	%	No.	%	No.	%
atmos.	ft.								
2	18,000	30 min.	23	0	0	0	0	0	0
3	27,000	30	10	0	0	0	0	0	0
4	34,000	30	8	0	0	0	0	0	0
5	38,000	30	8	0	0	0	0	0	0
6	42,000	30	10	0	0	0	0	0	0
7	45,000	30	10	5	50	0	0	0	0
8	48,000	30	10	7	70	1	10	3	30
9	51,000	30	19	11	58	3	27	11	100
10	53,000	30	15	14	93	3	^1	14	100
10	53,000	1	21	0	0	0	0	0	0
11	55,000	30	20	20	100	3	15	20	100
12	59,000	20	6	6	100	4	66	6	100
15	61,000	1	10	0	0	0	0	0	0
15	61,000	4	6	0	0	0	0	0	0
15	61,000	5	6	4	66	2	50	4	100
16.6	64,000	1	10	0	0	0	0	0	0
20	68,000	30 sec.	6	0	0	0	0	0	0
20	68,000	1 min.	18	9	50	7	77	9	100
20	68,000	2	9	4	47	1	25	4	100
20	68,000	3	9	6	66	2	33	6	100
20	68,000	4	12	10	83	4	40	10	100
20	68,000	5	6	6	100	1	17	6	100
23	71,000	1	6	4	66	0	0	4	100
30	76,000	10 sec.	16	0	0	0	0	0	0
30	76,000	30	12	8	66	4	50	8	100
30	76,000	50	12	12	100	0	0	12	100

Thus, of 74 rats, subjected for the same time-interval to demonstrably 'lethal' decompressions (7 to 11 atmospheres' differential), 57 (77%) succumbed. However, in only 10 animals (18%) was evidence of pulmonary hemorrhage seen at autopsy. On the other hand, in 48 of the rats (82%) killed by the decompression, there was definite aeroembolism (gas bubbles in the right heart and large vessels). It would hence appear that in these experiments, in which the animals were never anoxic, aeroembolism constituted the major lethal factor, rather than the physical expansion of the intrapulmonary gases.

This was further indicated by the fact that no deaths resulted in a series of 21

animals maintained under 11 atmospheres of pressure for only 1 minute prior to decompression (table 1). Moreover, since the average respiratory rate of sleeping and quiescent (resting) rats in the colony was found to be 82 and 115 per minute respectively (average of 50 determinations), one might safely assume the rather complete filling of the lungs with compressed air, even within 1 minute, and hence that all animals would be subjected to much the same degree of intrapulmonary gas expansion.

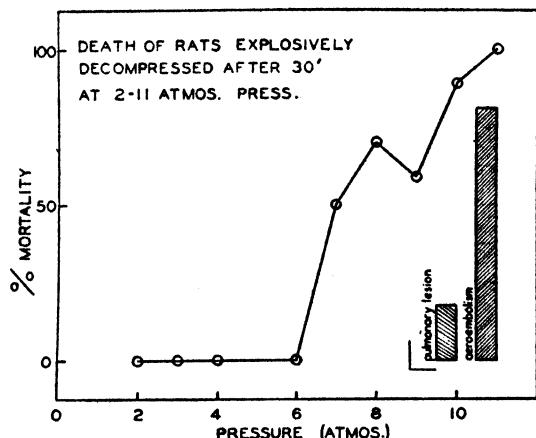


Fig. 1. MORTALITY RATE IN RATS explosively decompressed to ambient atmospheric conditions following exposure to 2-11 atmospheres of pressure.

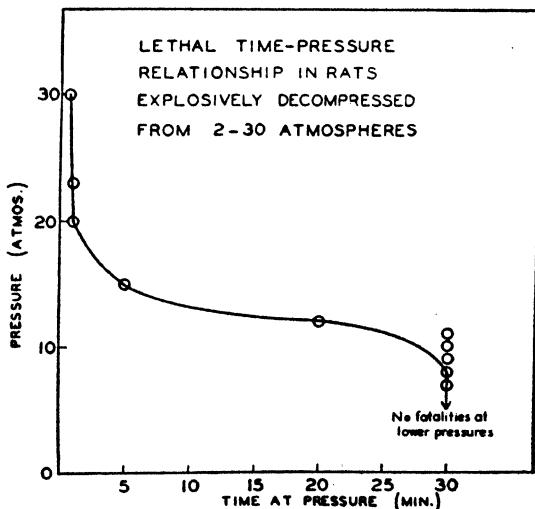


Fig. 2. TIME-PRESSURE RELATIONSHIPS productive of lethal explosive decompression injury in the rat.

In the hope of finding a pressure differential at which gas expansion *per se* might invariably result in lethal trauma (as evidenced by pulmonary hemorrhage), experiments were continued to include decompressions from even higher pressures. Such tests gave increasing evidence that time under pressure (and hence aeroembolism), rather than the expansion of intrapulmonary gases (fig. 2) constituted, in these experiments, the major factor in survival. All animals died following decompression

from 12 atmospheres (gas-expansion equivalent, 57,000 ft.), and exposures to 15 atmospheres for only 5 minutes resulted in the death of 4 of 6 rats on depression. However, all survived when time under pressure was reduced to 4 minutes, and when this interval was further reduced to 1 minute 20 animals tolerated decompression from 15 to 16.6 atmospheres of pressure without fatality.

Further evidence that time under pressure constituted the major lethal factor in this type of experiment was obtained in a series of 60 rats decompressed from 20 atmospheres (gas expansion equivalent, 68,000 ft.). The results are shown graphically in figure 3. Of these animals decompressed after 30 seconds to 5 minutes under pressure, 35 died, with pulmonary lesions in only 15 cases (43%), while all rats which succumbed gave marked evidence of aeroembolism. Throughout the study 'pulmonary lesion' was recorded whenever the smallest ecchymoses could be grossly detected. Hence, in a large percentage of animals in which hemorrhage was noted, the amount of pulmonary bleeding could not have contributed significantly to the death

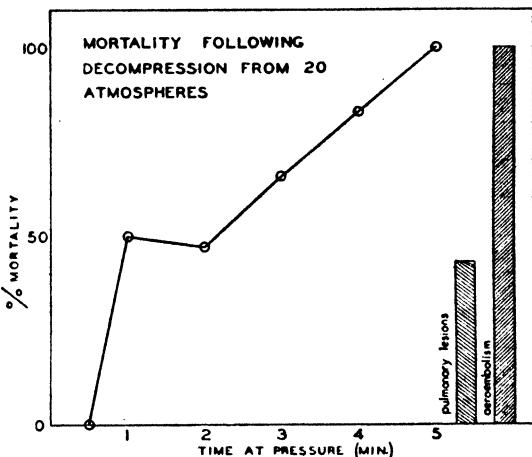


Fig. 3. MORTALITY RATE IN RATS explosively decompressed following exposure to 20 atmospheres of pressure for varying time intervals.

of the animal. Moreover, such pulmonary damage as was seen might, in many instances, have been due to the expansion of gas emboli within the smaller pulmonary vessels.

When decompressed after a 50-second exposure to 30 atmospheres all animals succumbed with marked aeroembolism. So great was the amount of gas within the heart that, on opening the thorax, an audible sound was, on occasion, produced by the cardiac contractions. All of the larger vessels exhibited emboli entirely blocking their lumina. However, no pulmonary bleeding was present in any of the 12 animals of this series. A reduction of the period of pressurization to 30 seconds resulted in the death of 8 to 12 rats, with pulmonary bleeding in 4 animals. All of the rats which succumbed showed marked aeroembolism. When time under pressure was further reduced to 10 seconds, all 16 rats survived. These results, like the foregoing, were interpreted as indicating that the physical expansion of the intrapulmonary air constituted a relatively minor contributing factor in causing death, and that in decompressions as here performed, i.e. in the absence of anoxia and following profound pressure changes, aeroembolism appeared to be the primary lethal agent.

### ANOXIA

Twenty rats placed in an atmosphere of nitrogen succumbed after an average interval of 1 minute, 17 seconds, when respiration ceased. In half of these animals, pulmonary damage was observed at autopsy. Such lesions varied from small, discrete ecchymoses to diffuse hemorrhage involving all of the pulmonary lobes. It was therefore apparent that asphyxia alone may result in pulmonary bleeding in the rat, indistinguishable grossly from that seen following explosive decompression, probably as a result of the extreme and convulsive dyspnea induced. It may be noted that asphyxia does not invariably produce such lesions, nor does explosive decompression, whether performed in the conventional manner (1) or as in the present study.

### DISCUSSION

It is of historical interest to note that Bert (2), in 1878, from a long series of studies on decompression injury concluded, "The physical phenomena amount to very little, even when the rapidity of the experiment should have increased their importance". Further, that 'the pulmonary ecchymoses' on release from pressure "mean nothing, because we find them in simple asphyxia, at normal pressure".

The pathology of explosive decompression injury is, in the main, at present quite well known (1, 3-8) and major differences of opinion among investigators appear to center about its chief etiological factors (6, 8-11). The determination of the principal causative agent or agents is of primary concern, since the design of any devices for the protection of aircrews from this hazard must depend on a knowledge of the mechanics and cause of injury.

Edelman *et al.* (3) are of the opinion that an increased intrapulmonary pressure is the primary etiological agent, and Whitehorn, Lein and Edelman have expressed a somewhat similar opinion (8). They considered aeroembolism to be a negligible hazard, following experiments with guinea pigs. Gelfan (9), on the other hand, considers anoxic anoxia to be the primary lethal agent. There is general agreement that rapid decompressions to simulated altitudes as great as 35,000 feet are entirely innocuous (1, 9).

It is interesting to note that lethal, simulated altitudes for the rat agree quite closely both in conventional studies and the present experiments. Thus Gelfan (9) found that rats succumb to anoxic anoxia at 52,000 feet (simulated), or higher while in the experiments described, a 93 per cent mortality occurred at a gas-expansion equivalent of 53,000 feet, and all animals died at the 55,000-foot equivalent on release from a 30-minute stay under pressure.

There is apparently little difference of opinion as to the value of rapid recompression (1, 10) to survival. This would obviously hold true whether the principal lethal factor be anoxic anoxia or aeroembolism, since both occur at sufficiently reduced pressures, and either condition may be corrected by recompression.

In experiments of conventional design, it is obvious that because of the comparatively small pressure differentials involved, aeroembolism would be of much rarer occurrence than in the experiments described. Quite recently, Hitchcock *et al.* (12), in experiments on human subjects found that when pressure oxygen breathing was employed, explosive decompressions to simulated altitudes up to 45,000 feet were well tolerated, without any evidence of bubble formation attributable to the explosive

decompression *per se*, although incapacitating bends and chokes did occur at extreme altitudes.

It would therefore appear that anoxic anoxia and aeroembolism constitute the major factors in causing death from explosive decompression, and that the physical expansion of intrapulmonary gases plays a relatively minor part in the etiology of injuries so produced.

#### SUMMARY

In experiments designed to eliminate the presence of anoxic anoxia, rats were explosively decompressed to normal atmospheric pressure after exposure to positive pressures of from 2 to 30 atmospheres for varying time intervals. Decompression from 2 to as much as 6 atmospheres (equivalent gas-expansion differential, 18,000 to 42,000 ft.) was well tolerated, and such decompressions may be considered to be entirely innocuous in the rat.

Of rats decompressed after 30-minute exposures to 7 to 11 atmospheres of pressure, 77 per cent died. Aeroembolism was present in 82 per cent of these animals, with pulmonary lesions in 18 per cent of cases. All animals succumbed following decompression from 11 atmospheres (gas expansion equivalent, 55,000 feet) when so maintained for 30 minutes prior to release of pressure. Without exception these animals exhibited gas emboli, while pulmonary hemorrhage was present in only 15 per cent.

All rats which died following explosive decompression from 20 atmospheres of pressure gave evidence of severe aeroembolism, while pulmonary lesions could be demonstrated in only 43 per cent of such animals. Decompression after a 50-second exposure to 30 atmospheres of pressure was invariably fatal. On the other hand, all survived this pressure change when time under pressure was reduced to 10 seconds. Pulmonary hemorrhage occurred in 50 per cent of rats dying from anoxic anoxia (nitrogen at normal atmospheric pressure).

These experiments indicate that anoxic anoxia and aeroembolism constitute the major factors in the etiology of explosive decompression injury, and that intrapulmonary gas expansion may be considered of relatively minor importance as a lethal agent.

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# MINIMAL EFFECTIVE DOSE OF INTRAVENOUSLY ADMINISTERED HISTAMINE IN PREGNANT AND NON-PREGNANT HUMAN BEINGS

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**I**N VIEW of the problems which surround the question of the physiological rôle of histamine, this study was undertaken to determine the minimal effective dose of histamine administered by rapid intravenous injection. Reported data are scattered and incomplete, although the minimal effective dose of histamine given by continuous intravenous infusion in man has been carefully worked out for gastric secretion (1), facial flush and changes in cerebral blood vessels (2).

In view of the rise of the histaminolytic property of the blood which has been reported during pregnancy (3), it seemed desirable to determine the minimal effective dose in a group of pregnant females as well, in an effort to find evidence for a physiological rôle for this elevated blood "histaminase".

Harmer and Harris (4) gave 20 micrograms of histamine base<sup>1</sup> intravenously on 12 occasions to an unspecified number of patients, all of whom experienced metallic taste and facial flush. Almost always they felt warm, and frequently, although less constantly, they experienced throbbing in the head. Best and McHenry (5) noted that 15 micrograms of histamine gave a metallic taste, tightness of the head and headache. Pickering and Hess (6) produced headache regularly with 40 micrograms of histamine. Weiss and co-workers (2) stated that the minimal intravenous dose of histamine that would exert changes in the cerebral and facial vessels was 7 micrograms of histamine, as measured by flush and rise in cerebro spinal fluid pressure.

Storch (7) chose 20 micrograms as the "active subthreshold dose" because he found that it did not produce headache in most instances in normal individuals.

Rothlin and Gundlach (8) obtained no gastric response in dogs by the rapid intravenous injection, in six instances, of doses of histamine varying from 0.05 mg to 0.4 mg.

## METHODS

This study employed 36 white adult subjects: 12 normal males, 14 non-pregnant females, and 10 pregnant females in the second and third trimesters of pregnancy.

Histamine dihydrochloride, diluted with physiologic saline so that 1 ml. of solution contained 20 micrograms of histamine base, was administered intravenously as rapidly as possible through a 23-gauge needle in volumes which ranged from 0.2 ml to 1.5 ml. The subjects were seated comfortably in a good light. To avoid the distracting influence of the venipuncture the intravenous injection was made a short time after the needle was introduced into the vein following the release of the tourniquet. The subjects were unaware for the most part of the expected reactions, and

Received for publication February 15, 1949.

<sup>1</sup> All doses of histamine are calculated as histamine base.

were simply asked to describe in detail all their sensations as they occurred, while they were being observed for facial flush.

The rapid intravenous injection of histamine produced the responses which have been described in detail by others. A metallic taste was perceived usually 20 to 30 seconds after injection, followed by a marked facial and neck flush (25-35 seconds after injection). This was usually accompanied by sensations of warmth over the entire face and forehead. Between 40 and 60 seconds after injection, dizziness, lightheadedness and vertigo were felt, associated with the sensations of pressure, throbbing and ache or pain within the head. The headache or pain produced by the doses employed reached a peak of intensity within 1 to 1½ minutes, and subsided promptly within 2 to 3 minutes after injection.

After some preliminary observations the initial dose employed was usually 4 or 10 micrograms. These were randomized and repetition of intravenous doses was done no sooner than at 15-minute intervals, or after all symptoms had disappeared. In 30 subjects only two different doses were required to elicit clear-cut differences of response; in 6 subjects three different doses were required, although threshold doses for every type of response were not obtained in every individual, in the range of doses tested.

Five control subjects were retested one week following the original observation. There was complete reproduction of their original responses at the same dose levels.

#### RESULTS

From the results summarized in table 1 and figure 1, it is apparent that for any given individual the minimal effective dose of histamine is not the same for all of the phenomena studied. It is apparent also that doses of histamine of the range of 2 to 4 micrograms are capable of stimulating taste receptors and effecting changes in facial and cerebral blood vessels in 25 per cent of the subjects studied, and that doses of 10 micrograms effected these changes in 75 per cent of the subjects.

Further, the range of minimal effective doses of histamine in the group of pregnant subjects is within the range of the minimal effective doses of the control group.

#### DISCUSSION

The changes induced by histamine in this study fall into three groups : 1) changes in taste receptors, 2) changes in facial vessels manifested by flush and sensations of warmth and 3) accepting Pickering's analysis (6), changes in cerebral blood vessels manifested by sensation of dizziness, and headache or pain.

Although our values are somewhat smaller, our findings are in keeping with those reported by Weiss *et al.* (2), who studied cerebral vascular changes by the rise of cerebrospinal fluid pressure. They clearly indicate the extreme sensitivity of these vessels. With headache and flush as our criteria we were unable to note any marked difference between cerebral and facial blood vessels.

It is of interest in this connection that the minimal effective dose of histamine administered by continuous infusion in man is 0.004  $\mu\text{g}/\text{kg}/\text{min}$ . for gastric secretion; 0.001-0.04  $\mu\text{g}/\text{kg}/\text{min}$ . for facial flush, and 0.02-0.05  $\mu\text{g}/\text{kg}/\text{min}$ . for headache (1, 2).

The finding that the minimal effective dose of histamine is the same for the

pregnant group as for the control group is of considerable interest in view of the marked rise in histaminolytic power of the blood in pregnancy. Ahlmark (3) in a

TABLE I. MINIMAL EFFECTIVE DOSE OF HISTAMINE IN MICROGRAMS OF BASE

SUBJECT	SEX	TASTE	FLUSH	WARMTH	DIZZINESS OR VERTIGO	HEAD PAIN OR HEADACHE
I	M	10	10	20	20	20
2 <sup>1</sup>	M	10	10	10	20	20
3 <sup>1</sup>	M	4	4	10	10	10
4 <sup>1</sup>	M	10	10	10	10	10
5	M					
6	M		4	10	10	4
7	M		10		4	
8	M		4	10	4	10
9	M	4	10	4		4
10	M	10	10	10	20	10
11	M	10	10	10	10	10
12	M	10	15			
13 <sup>1</sup>	F	2	4	2	2	2
14	F	10	10	10	10	10
15	F		10	4		10
16	F	4	10	10	10	10
17	F	10	10	4	4	4
18	F	4	4	4	4	20
19	F	20	10	10	10	20
20	F	10	10	4	10	10
21	F	30	20	10	20	30
22	F	10	10			10
23	F	10	4	4		10
24	F	4	10	10	4	4
25	F	4	4	10	10	4
26	F	10	4	4		
MONTH OF PREGNANCY		TASTE	FLUSH	WARMTH	DIZZINESS OR VERTIGO	HEAD PAIN OR HEADACHE
27	9	10		10		10
28	8	10		4	4	4
29	7	4	10	10	4	10
30	8	10	10	10	10	10
31	8	4	10	10	10	15
32	7	10	10	10	10	10
33	6	4	4	4	10	10
34	6	4	10	10	10	10
35	6	15	15	15	10	15
36	5	10	10	10	10	4

<sup>1</sup> Retested subjects.

recent and careful study has reported that during the second and third trimesters, on the average, 1 ml. of plasma of a pregnant woman will inactivate 5 micrograms of histamine per hour. This is equivalent to 0.2 Winthrop unit of histaminase per ml. of blood.

It has recently been shown in the dog that the intravenous injection of 20 units of hog kidney histaminase per kilogram of body weight, which is equivalent to 0.4 units per ml. of blood and which is capable of inhibiting the minimal effective gastric secretory dose of histamine, does not inhibit the vasodepressor response of a minimal effective dose of histamine given intravenously (9). This minimal effective vasodepressor dose is six times larger than the minimal effective dose for gastric secretion (1). However, larger doses of histaminase (1000 u/gm.) definitely inhibited the vasodepressor action of histamine in cats (9).

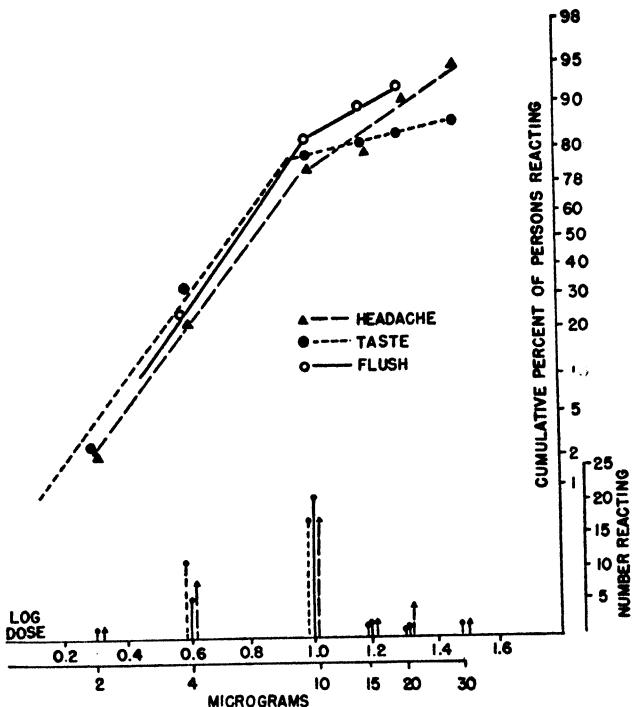


Fig. 1. VERTICAL LINES SHOW FREQUENCY DISTRIBUTION of the number of reactors at each dose level. Sloping lines show the cumulative percentage of persons reacting at the various dose levels. The percentage scale is in probability units so that a straight line would result if there were a normal frequency distribution. The fact that the cumulative percentage lines are truncated indicates an abnormal frequency distribution.

It should be emphasized that the speed with which an enzyme acts depends, among other factors, upon the concentration of the enzyme. The failure of the histaminase of pregnancy to elevate the threshold for the vascular responses to intravenously injected histamine reflects an inadequate concentration rather than slowness of reaction. That such enzymatic actions may occur within a matter of milliseconds in the presence of adequate concentrations of enzyme has been shown by Nachmansohn for the cholinesterase of neural tissue (10). It is also possible that the blood 'histaminase' of pregnancy may be capable of neutralizing the minimal effective

gastric secretory dose, although by the method of rapid intravenous injection of histamine no elevation of threshold was elicited in the pregnant group.

#### SUMMARY

Two to four micrograms of histamine base injected rapidly intravenously stimulated taste receptors and effected changes in facial and cerebral blood vessels in 25 per cent of the subjects tested. Ten micrograms of histamine effected these changes in 75 per cent of subjects tested. The range of minimal effective doses of histamine given rapidly intravenously is the same for pregnant and non-pregnant subjects.

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# WORK PERFORMANCE OF NORMAL RATS GIVEN CONTINUOUS INJECTIONS OF ADRENAL CORTEX EXTRACTS

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IT HAS been established that the resistance of non-adrenalectomized animals to certain types of stress can be increased by the administration of adrenal cortex extracts and steroids. However, the known conditions where these hormones benefit non-adrenalectomized animals are few and the results of most studies of this general problem have been negative. The field has not been fully explored and some claims of positive results will require confirmation before they become acceptable.

In the early years following the isolation of potent adrenal cortex extracts there were a number of reports that normal animals were made more resistant to fatigue by the administration of adrenal cortex extracts. The reports (1-3) of a synergistic effect of adrenal cortex extract and ascorbic acid upon the work performance of non-adrenalectomized rats prompted one of us (*D. J. I.*) to carry out an extensive series of studies (unpublished) of this problem during 1936 to 1938. Adrenal cortex extracts and ascorbic acid were tested separately and in combination upon the ability of normal rats to work in the following situations: 1) the swimming time of the unanesthetized rat weighted with 10 gm. and weighted with 20 gm.; 2) the running time of the unanesthetized rat in a motor-driven revolving cage; 3) the work of the gastrocnemius muscle of the anesthetized rat during 120 hours of faradic stimulation. Amounts of adrenal cortex extracts up to 3 cc. per day (75 gm. of gland per cc.) were tested in each study. All of the results were negative.

More recently we have developed an apparatus for the continuous injection of hormones and a modification of the muscle-work test (4) which permits the activation of all of the musculature of both back limbs. This procedure is a severe stress which rapidly produces hypoglycemia in normal rats unless glucose is administered. Some working normal animals develop hypoglycemic convulsions and die within 24 hours. Since adrenal cortex extract tends to stimulate gluconeogenesis and to conserve carbohydrate in both adrenally insufficient and normal animals, at least under resting conditions, and since the cortical hormone requirement of the adrenally insufficient rat is very high under these conditions (5) we have reinvestigated the effect of continuous injections of adrenal cortex extract upon the work performance and survival of the non-adrenalectomized rat. The results were negative.

## METHODS

Male rats of the Sprague-Dawley strain which weighed  $200 \pm 2$  gm. were used. These animals were free from parasites and infections. The diet was Archer Dog

Pellets. The procedures used for the stimulation of muscle were according to Ingle (4) with the following modifications. A Nerve Stimulator, Model B, Upjohn, was used to stimulate muscle at the rate of 5 times per second. The duration of each pulse was 20 milliseconds and the intensity was 20 milliamperes. An electrode was placed on the lower tibia of the left back leg and the second electrode on the contralateral back foot, thereby activating all of the musculature of both hind legs. The gastrocnemius muscle of the left hind leg was weighted with 100 gm. The distance that the weight was lifted was registered on automatic work recorders. Each recorder revolution represented approximately 400 gm.-cm. of work.

A solution of 0.9 per cent sodium chloride with and without beef adrenal extract was either given by subcutaneous injection or it was infused into the jugular vein at a constant rate by means of a constant injection apparatus which simultaneously delivered fluid from each of 12 syringes at the rate of 20 cc. in 24 hours. The adrenal cortex extract represented 40 gm. of beef adrenal extract per cc. and was free from alcohol. All doses of ACE were diluted to 20 cc. with saline. Temperature was constant at  $28 \pm 0.5^{\circ}$  C.

The animals were anesthetized with phenobarbital sodium and cyclopentyl sodium (4). They were subjected to the work tests as soon as surgical anesthesia was attained. Stimulation was continued until the muscle ceased to respond or for 24 hours.

#### EXPERIMENTS AND RESULTS

The data of these experiments are summarized in table 1. Twelve pairs of rats were represented in each experimental group. The animals treated with ACE and their controls were always tested simultaneously.

Three groups of rats were tested without prior fasting. The doses of ACE were 5, 10 and 20 cc/rat/24 hours. The solutions were given by subcutaneous injection.

Two groups of rats were fasted for 24 hours prior to the beginning of the work test. The amounts of ACE were 10 and 20 cc/rat/24 hours. The solutions were given by subcutaneous injection.

One group of rats was fasted for 24 hours prior to the beginning of the work test. One rat of each pair received 20 cc. of ACE per 24 hours by intravenous injection.

Two groups of rats were fasted 32 hours prior to the beginning of the work test. One rat of each pair was given 2 cc. of ACE by subcutaneous injection at 8, 6, 4 and 2 hours prior to the beginning of the work test. The control animals received saline injections. The experimental animals received 20 cc. of ACE/rat/24 hours during the work test. The results of these two groups are combined together in table 1.

There was no significant difference between the average amounts of work performed by rats which received ACE and their controls which received saline in any of the experimental groups. The average amount of work done by each of the 96 rats which received ACE was 19,470 recorder revolutions and the average for all of the control animals was 19,465.

There was no tendency for the administration of ACE to reduce the number of deaths from hypoglycemia during work. Among the 36 pairs of non-fasted rats 4 of the ACE rats died and 6 of the saline controls died. Among the 36 pairs of rats

which fasted 24 hours, 11 of the ACE rats died and 10 of the saline controls died. Among the 24 pairs of rats which fasted for 32 hours, 8 of the ACE (pre-treated) rats died and 4 of the saline controls died.

One group of rats was fasted for 32 hours and one rat of each pair was given 2 cc. of ACE at 24, 26, 28 and 30 hours by subcutaneous injection. The control animals were given equal volumes of saline. At 32 hours the animals were anesthetized with cyclopal and the liver glycogen was determined according to Pabst *et al.* (6). The

TABLE I. WORK PERFORMANCE OF NORMAL RATS GIVEN CONTINUOUS INJECTIONS OF ACE (BEEF ADRENAL CORTEX EXTRACT)

ACE PER 24 HRS. cc.	ROUTE OF INJECTION	FAST PRIOR TO WORK, HRS.	PRE-TREAT. PRIOR TO WORK, HRS.	NUMBER PAIRS OF RATS	TOTAL WORK <sup>1</sup> , AVERAGES AND RANGE	
					ACE	Control
5	SQ	0	0	12	24940 15472-31014	22132 5109-37875
10	SQ	0	0	12	25934 10478-40143	23865 5937-37333
20	SQ	0	0	12	23519 10869-33353	24780 10824-36001
10	SQ	24	0	12	12506 2179-23271	15259 4420-25398
20	SQ	24	0	12	16485 5711-31720	14124 7461-24670
20	IV	24	0	12	16745 8142-25450	18904 9996-24808
20	IV	32	8	24	17816 5236-26490	18329 7285-25420

<sup>1</sup> Work is expressed as recorder revolutions. Each recorder revolution represents approximately 400 gram-centimeters of work.

Average for all ACE, 19,470; for all controls, 19,465.

average amount of glycogen in the livers of the ACE rats was 56.7 mg. and the average amount in the livers of the controls was 5.33 mg.

#### DISCUSSION

We have been unable to show that the work performance of the non-adrenalec-tomized rat is improved by the administration of large amounts of adrenal cortex extract. The secretory activity of the adrenal cortices of the intact rat is apparently capable of meeting the needs of the organism under these conditions. However, it has been shown that the removal of one intact adrenal does limit the ability of the rat to work (5).

Since the normal rat develops hypoglycemia and may die under the stress of work, it was anticipated that the administration of ACE should offer some protection against collapse from hypoglycemia just as the resistance of the normal rat (7) to insulin can be raised by the cortical hormones. This did not occur even when the rats were fasted prior to work. Pretreatment with ACE did increase the level of liver glycogen ten-fold but the actual energy value of this increase in liver glycogen is negligible when the carbohydrate requirement of the working rat is considered (8).

#### SUMMARY

Normal rats were subjected to the faradic stimulation of both hind legs at a rate of 5 times per second until death occurred or for a period of 24 hours. The continuous subcutaneous and intravenous administration of adrenal cortex extract in amounts of 5, 10 and 20 cc. of beef adrenal extract/rat/24 hours failed to improve the work performance of either fasted or non-fasted rats. The pretreatment of the fasted rat for 8 hours prior to the beginning of the work test failed to either improve the work performance of the animals or to protect against collapse (hypoglycemic), although this procedure caused a ten-fold increase in liver glycogen in a similar series of animals.

We wish to express our appreciation to Miss Joan E. Wilhelm who carried out the determinations of liver glycogen in this study.

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# ACUTE HYPOTHERMIA IN GUINEA PIGS<sup>1</sup>

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MOST investigators of hypothermia in mammals agree that anoxia plays a significant rôle, if not the dominant one, in death by cold. Although the literature contains no conclusive proof that the diminution in oxygen consumption during hypothermia is relatively greater than the concomitant reduction in the oxygen requirement, much evidence supports this thesis. The major differences of opinion among investigators of this problem arise in defining the mechanism or mechanisms by which anoxia occurs.

Pioneer experimenters were impressed with the profound depression of respiration, leading eventually to apnea often before cardiac standstill. Their observations have led to the conclusion that hypothermia is responsible for a direct inhibition of the respiratory reflexes and that the ensuing anoxia is predominantly anoxic in type. In this regard the apparently beneficial influence of artificial respiration is often cited. On the other hand, Crismon (1) believes that a stagnant anoxia is the critical factor, arising from a suppression of cardiac output as evidenced by a profound slowing of heart rate and terminal drop in arterial blood pressure; in his opinion apnea is only secondary to cardiac failure. German experiments summarized by Alexander (2) support a similar thesis. Werz (3) has furnished evidence that a lowered rate of dissociation of oxyhemoglobin may be a limiting factor in the availability of oxygen for tissue metabolism. Dill and Forbes (4) have calculated that in spite of presumably high alveolar O<sub>2</sub> pressures the arterial oxygen tension was significantly reduced by cooling their human subjects; they suggest that slow diffusion of oxygen across the alveolar walls gives rise to anoxia. Denying the crucial rôle of anoxia, Grosse-Brockhoff and Schoedel (5) stress a direct hypothermic paralysis of medullary centers and an impairment of processes of excitation and conduction in the heart.

The data reported here represent diverse physiological observations on unanesthetized guinea pigs during and following cooling in an air jacket surrounded by ice water. These measurements include responses of colonic temperature, heart rate, ventilation rate (minute respiratory volume), oxygen consumption and carbon dioxide output. Attempts are made to correlate these physiologic indices with the observed behavior of each animal and with its success or failure to survive these acute and severe reductions of body temperature.

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Received for publication February 9, 1949.

<sup>1</sup> This study was aided by a contract between the Aeromedical Laboratory, U. S. Air Forces, and the University of Rochester.

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## PROCEDURE

Guinea pigs of both sexes ranged in body weight from 250 to 380 gm. Each experimental animal was carefully restrained by adhesive tapes attached to a wire mesh which formed an open cylinder loosely encircling the torso. The animal and its restraints were then inserted into a large surgical rubber glove which was suspended vertically and lowered into an ice water bath so that only the open end of the glove and the animal's head remained above the water surface. By this technique the animal's fur remained dry throughout (except on the perineum due to urination). Because an appreciable but variable amount of air was trapped in the glove during the immersions, rates of cooling were slower and presumably more variable than those that might be observed with direct immersion; perhaps the dry fur lent more uniformity to subsequent rates of rewarming in air. After a severe reduction in body temperature (to 17-21°C.), the water bath and glove were removed, and the animal was allowed to rearm in still air (usually at either 25° or 27.5°C.). Although many animals were maintained in a vertical posture for 6 to 8 hours, no significant changes in rectal temperature, heart rate, ventilation rate or oxygen consumption could be detected when the animal was restored to a prone position before or after the immersion.

An index of the temperature of deep tissues was obtained with a calibrated constantan-iron thermocouple inserted per rectum so that the junction lay within the colon at a depth of 6 to 8 cm. (approximately at the level of the hilus of the left kidney). When the junction at this depth was moved 1 or 2 cm. in either direction, the recorded temperature did not change by more than  $\pm 0.1^{\circ}\text{C}$ . Although the junction was exposed, both insulated wires from it were encased in a soft rubber catheter, and the entire unit was firmly held in place throughout the entire period of cooling and rewarming. A recording potentiometer automatically registered the thermoelectric voltage every 3 minutes throughout the test. The sensitivity of the apparatus was  $\pm 0.1^{\circ}\text{C}$ .

In 9 tests, needle electrodes connected to a standard string galvanometer were inserted subcutaneously over the precordium and in the groin. Electrocardiograms were photographed periodically during both cooling and recovery. Heart rates measured from these records were further supplemented at low rates by counting directly the oscillations of the galvanometer string.

The measurement of ventilation rate (minute respiratory volume) in small unanesthetized mammals offers several technical problems. A respiratory mask was eventually constructed which proved air-tight on all guinea pigs tested. The crucial feature of the mask was a broad tight cuff of dental dam acting both as an air seal around the neck and as a harness to hold the mask in place. Around this cuff was wrapped a long strip of dental dam about 5 cm. wide; it extended well over the animal's shoulders. Small rubber flap valves connected to the mask offered negligible resistance to both inspiration and expiration. Generally room air entered the inspiratory valve but occasionally pure oxygen was introduced. Expired air was collected in a small spirometer (capacity 1 liter) and excursions of the spirometer drum were recorded continuously by a pen on a moving paper belt. As calculated from the slopes of these lines, our values of ventilation rate are dependable within 5 to 10 ml/min. Each recorded value of the minute respiratory volume represents the average response of several minutes (generally 2 minutes but as long as 10 minutes during the respiratory depression of severe hypothermia). Volumes of expired air were measured at 25°C. and, in general, ventilation rates have not been corrected from that to body temperature.

Ventilation rates measured prior to cooling appeared inappropriately high, even after acknowledging that these unanesthetized animals were not in a truly resting state as judged by their intermittent but persistent struggling. Thus initial values in various experiments ranged from 360 to 520 ml/min. (average 420). Although the mask and valves were designed so that the dead space was only a few ml., any dead space in the apparatus is made significant by the inevitably rapid breathing and low tidal volume of these small mammals. Although this respiratory dead space must have varied from test to test it remained relatively constant in any one experiment. Therefore, relative changes in the observed ventilation rate during the course of hypothermia are valid, even though absolute values are of doubtful physiological significance. For this reason, all ventilation rates are here reported in percentage of the initial rate prior to immersion, a value obtained by averaging several observations over a period of at least one hour. These duplicate determinations seldom varied from their mean by more than 5 per cent.

In 6 tests, samples of expired air were taken periodically from the spirometer and analyzed for

oxygen and carbon dioxide. The gas analyzer, described by Rahn *et al.* (6), employs a Pauling oxygen tensimeter and a Cambridge thermoconductivity meter for carbon dioxide. Because of dead space within the mask and particularly because of a common pathway from the mask to inspiratory and expiratory valves, samples of expired air were relatively high in oxygen (e.g., 18.7%) and low in carbon dioxide (e.g., 1.9%). It is apparent, however, that absolute values of oxygen consumption and carbon dioxide production calculated from these analyses are not invalidated by such dead space. Values reported are expressed in mm<sup>3</sup>/min/gm. of body weight, and they are undoubtedly dependable within  $\pm 10$  per cent.

### RESULTS

*Behavior.* The average pre-immersion colonic temperature was 38.1°C. (standard deviation  $\pm 0.75$ ). Within 3 to 6 minutes of exposure to cold, the body temperature invariably fell; between 35 and 22°C., the rate of cooling was sensibly linear (0.2 to 0.3°C. per min). Shortly after the immersion, all animals intensified their struggling. Respiratory and ventilation rates accelerated during this initial period. Some animals consistently vocalized, others never. Gnawing was a constant feature of their efforts to escape. Tearing, salivating and chewing were prominent on occasion. Shivering as such was not invariably present, the increased motor activity often being of a jerky convulsive character. Such purposeful activity generally ceased at about 26°C., shortly after the beginning of a gradual fall in ventilation. When present, shivering disappeared a few degrees lower; it was often superseded by a slow rhythmic tensing of the limbs. By 20°C., all extraneous motor activity had generally disappeared; muscles became flaccid, respirations were slow, shallow and gasping (though seldom irregular). There were no detectable responses to cutaneous stimulation.

A critical but sublethal exposure was intended, but no single criterion proved to be a reliable signal for terminating the cooling. As judged in these tests, the colonic temperature itself was not crucial. Some animals survived briefly at 17°, others succumbed at temperatures between 20° and 21°. As demonstrated later, data on heart rates and ventilation rates are valuable but not predictive, since even apnea and ventricular fibrillation may be reversible. The decision to terminate an immersion remained arbitrary. Minimal temperatures ranged from 17.1 to 21.8°, averaging 19.3° C. When the guinea pigs were restored to still air at 25°C. (or 27.5°), colonic temperatures continued to fall for 9 to 12 minutes and then rose slowly.

At this point, heat production was generally so low that the living animal warmed little faster than the dead one. Gradually heat production assumed the major rôle in repaying the thermal debt. Relative to the motor activity during cooling, rewarming was a peaceful operation. A few animals were restored to normal colonic temperatures without displaying any detectable shivering or struggling. Generally, however, both appeared, though only at higher temperatures than those at which they had ceased during cooling. However intense this activity during the recovery process, it generally lessened after 3 to 4 hours. Simultaneously, the ventilation rate and oxygen consumption fell. As a result the colonic temperature tended to level off, often far short of normal levels.

*Heart Rates and Electrocardiograms.* Figure 1 illustrates the progressive fall in heart rate which accompanied cooling (9 tests). It is noteworthy that cardiac slow-

ing appeared as soon as there was a detectable fall in colonic temperature, in spite of the frenzied physical exertion which characterized the early stages of cooling. To be sure, the rate of fall was slightly slower during the initial decrement of 2 to 3° C. than thereafter. Between 36 and 23°C., the relationship between colonic temperature and heart rate was sensibly linear. The regression line (obtained by the method of least squares) had a formula  $y = + 17.5x - 312$  (where  $y$  = heart beats per min. and  $x$  = colonic temp. in °C.). Comparable data on adult anesthetized rats reveal a slope of 15 beats per minute per °C. (1); and on unanesthetized rats 19 beats per minute per °C. (7); but at all temperatures, rates in rats are 50 to 75 beats per minute higher than in guinea pigs. The latter difference may be a significant factor in the greater susceptibility of guinea pigs to a hypothermic death.

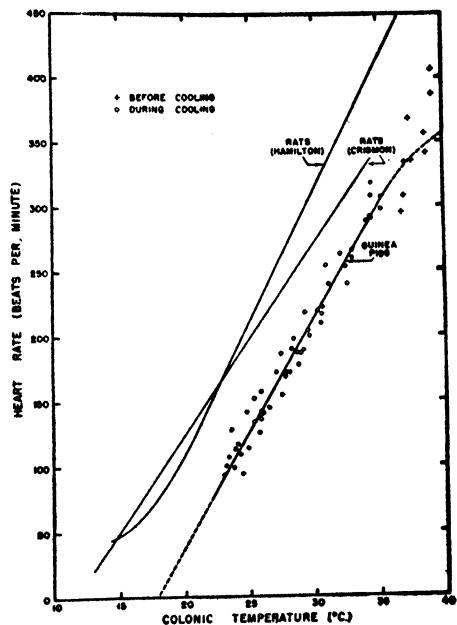


Fig. 1. RELATIONSHIP BETWEEN HEART RATE and colonic temperature in 9 guinea pigs during cooling between 35° and 23° C. Lines for unanesthetized rats after Hamilton (7), for anesthetized rats after Crismon (1).

The high correlation illustrated in figure 1 suggests that the body temperature is a direct determinant of heart rate. The relationship appears to be independent of the rate of cooling. When the rectal temperature was held at a constant low level (21.6°C., fig. 6), the heart rate remained relatively stable for several hours. Below 20 to 22°C., however, the heart rate became erratic. In some tests, the rate leveled off and in others it dropped precipitously. At rates below 70 beats per minute, the rhythm generally became irregular. These arrhythmias usually appeared to be of sinus or nodal origin. Once established, the arrhythmia generally persisted until a rise in body temperature had accelerated the rate to well over 100. In one test (fig. 5), an irregular ventricular rate of not over 13 beats per minute persisted for 30 minutes and included short episodes of ventricular fibrillation, after which a normal sinus rhythm returned as the animal warmed in air.

Because of muscle action potentials, no detailed electrocardiographic analysis was possible. Data on anesthetized rats have been well summarized by Crismon (1). In his studies abnormalities associated with cooling included prolongations of the PR, QRS and ST intervals, voltage increases, changes in the contour of the P- and T-waves, elevations of the ST segment, production of various degrees of A-V block, sinus and nodal arrhythmias, complete disappearance of P-waves, and ectopic ventricular beats. Many of these phenomena were identifiable in our records. A slow bizarre pattern diagnostic of ventricular fibrillation, seen once, has not been previously described in hypothermia. The mechanism of origin of these abnormalities has recently been investigated by such techniques as vagotomy (5), local heating of the heart (8), artificial respiration (1), and injections of atropine, glucose, calcium and cardiac glycosides (9, 10). Crismon (8) believes that terminal arterial hypotension in cooled rats is due to this bradycardia (a direct temperature action on the pace-makers and conducting mechanism) and to impaired ventricular emptying (an expression of inadequate myocardial nutrition). According to him, circulatory collapse is the critical feature of hypothermic death in rats. Data on guinea pigs are not complete enough to appraise the functional significance of the electrocardiographic findings.

All investigators agree that an animal which has survived a severe cooling carries no stigma of residual cardiac damage. In the present study, too, electrocardiograms returned to normal. In the early phases of recovery, arrhythmias disappeared as the rate rose. Although rewarming was 4 to 5 times slower than cooling, the correlation between heart rate and colonic temperature was essentially the same whether the temperature was rising or falling. A tendency was noted, however, for the rate at a given temperature to be slightly higher during the cooling. This may be due to the probability that heart temperature was slightly higher than colonic temperature during cooling and conversely during rewarming. If this interpretation is correct, the two temperatures never differed by more than 0.5°C.

*Ventilation Rate and Oxygen Consumption.* Figure 2 demonstrates the relative changes in ventilation rate during the cooling of 15 guinea pigs (dark line is the 'average'). Within 1 to 2 minutes of the immersion, before any drop in deep body temperature, struggling increased and hyperventilation began, presumably due to cutaneous stimulation. Maximal ventilation rates, however, did not appear until an appreciable reduction in colonic temperature (average 35°C.), and amounted to 30 to 100 per cent above pre-immersion values (average 70%). Below 35°C. ventilation rates declined steadily, reaching control values at 26° to 28° C. and approaching zero usually below 20°C. The variability shown in figure 2 is unexplained, but those animals with rates well below the 'average' line died in spite of their relatively mild exposures. Apnea, however, does not necessarily preclude a spontaneous recovery provided rewarming is commenced promptly after the cessation of breathing. For example, while in room air immediately after its immersion, one animal was apparently apneic for 20 minutes, during which time its colonic temperature rose 1.2°C. At this point, breathing reappeared spontaneously, and its recovery was subsequently uneventful.

The literature contains several detailed analyses of the relation between the oxygen consumption of guinea pigs (and other small mammals) and the envi-

ronmental air temperature (11), but a systematic comparison with the body temperature in deep hypothermia was not found. In the present study values of oxygen consumption (fig. 3) paralleled those of ventilation rate. The average initial rate of  $20.8 \text{ mm}^3/\text{min/gm}$ . is 50 per cent higher than values reported for resting metabolism (11, 12), a difference readily ascribable to struggling even before the immersion. Maximal rates of 33 to  $38 \text{ mm}^3/\text{min/gm}$ . (2-3 times estimated basal) occurred at colonic temperatures of 35 to  $36^\circ\text{C}$ . These values are 90 per cent above the peak metabolism observed by Herrington (11) when he exposed guinea pigs to cold air at  $14^\circ\text{C}$ . At temperatures below 27 to  $29^\circ\text{C}$ ., oxygen consumption dropped below pre-cooling rates and thereafter declined steadily along with the

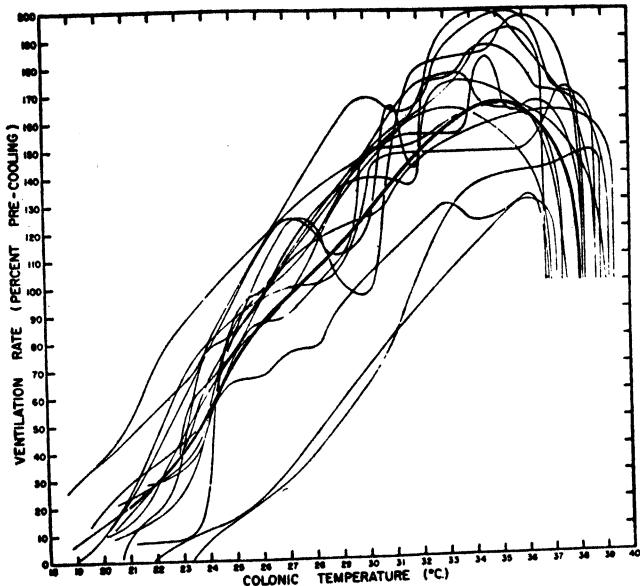


Fig. 2. RELATIONSHIP BETWEEN VENTILATION RATE and colonic temperature during cooling of 15 guinea pigs. Dark line is the line of 'averages' in the ordinate.

ventilation rate. With anesthetized dogs immersed in cold water, Grosse-Brockhoff and Schoedel (5) observed maximal rates of oxygen consumption at rectal temperatures of  $32$ - $33^\circ\text{C}$ .; these rates were 100 to 300 per cent above initial values. In men with rectal temperatures of  $35^\circ\text{C}$ ., Dill and Forbes (4) observed metabolic rates three times basal.

It is instructive to compare rates of ventilation and of oxygen consumption. The oxygen ventilatory equivalent (ventilation rate per 100 ml. oxygen uptake) is a convenient index for this purpose. In calculating this ratio, volumes of expired air (ventilation rate) were first converted to those at colonic temperatures (i.e., estimated lung temperature). Dill and Forbes (4) observed high ventilatory equivalents in human beings (schizophrenic patients) cooled to rectal temperature of about  $30^\circ\text{C}$ ., at which point oxygen consumptions were still above basal. Figure 4 demonstrates

that in guinea pigs this ratio remained high even during the severe respiratory and metabolic depression of deep hypothermia. Since the ventilation rate remained high relative to the oxygen consumption even to the stage of terminal apnea, the progressive fall in oxygen consumption cannot be ascribed to a paralysis of external respiration.

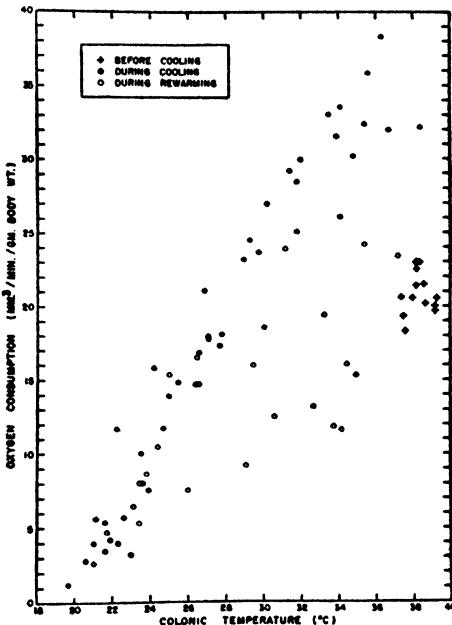


Fig. 3. RELATIONSHIP BETWEEN OXYGEN CONSUMPTION ( $\text{ml}^3/\text{min}/\text{gm. body wt.}$ ) and colonic temperature before, during and after cooling of 6 guinea pigs.

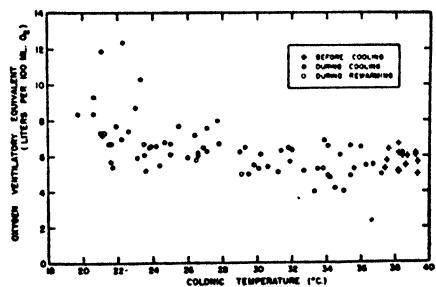


Fig. 4. RELATIONSHIP BETWEEN OXYGEN VENTILATORY EQUIVALENT ( $1/100 \text{ ml. oxygen consumed}$ ) and colonic temperature before, during and after cooling of 6 guinea pigs.

In mild prolonged hypothermia in man, Dill and Forbes (4) report a fall in respiratory quotient (RQ) and suggest that a depletion of liver glycogen is responsible. In the present study, a correlation between RQ and colonic temperature cannot be demonstrated unequivocally. A trend of questionable significance consisted of an elevation of RQ during the early phases of cooling, and a return toward the pre-cooling average (0.85) in deep hypothermia. Late in recovery, the RQ distinctly

fell as the body temperature returned toward normal. These shifts may indicate no more than a depletion of carbohydrate reserves.

During rewarming the ventilation rate and oxygen uptake slowly rose, but both rates were lower than those during cooling (at the same colonic temperature). The difference is probably related in part to the development of fatigue and decrease in struggling. Except for heart rate, vital signs during rewarming varied widely among the tests. The oxygen consumption (fig. 3) and ventilation rate may rise so slowly that pre-cooling values are never reached, may return to pre-cooling values in mid-recovery (about 27°C.) and then stabilize as the temperature continues to rise, or may climb late in mid-recovery to slightly above pre-cooling rates and then return to initial values. Of course, those animals with the most intense metabolism restored normal body temperatures fastest. Among the others, late fatalities were frequent, as demonstrated below. Early in the rewarming, however, it was impossible to predict which of the above metabolic patterns would develop. A consistent trend during recovery was a steady decline in the oxygen ventilatory equivalent (fig. 4) to levels 10 to 40 per cent below pre-cooling values. Data are inadequate to appraise the functional significance of this decline or to decide whether it is less marked among survivors than among those who die in mid-recovery.

Figure 5 summarizes observations on the colonic temperature, heart rate, ventilation rate, oxygen consumption and oxygen ventilatory equivalent during a typical cooling and the early phases of rewarming.

*Survival.* In analyzing survival, it is convenient to establish four categories of performance as in table 1. Insofar as sampled in these tests, sex and body weight did not condition performance. Acclimatization did not modify the average result since only rarely was the same animal used in more than one experiment. The average minimal temperatures are recorded in the table only to demonstrate that this factor did not determine the outcome, since these averages do not differ appreciably.

Immediate deaths (class A in the table) are those which occurred during cooling or shortly thereafter (within 1°C. of the minimum). As was intended, such fatalities were few. Even if the samples were larger, such data could not be interpreted in terms of a median lethal temperature, because the duration of exposure at any one temperature was not controlled and death cannot be associated with any particular colonic temperature when the latter is changing rapidly. Finally a hypothermic death can be detected only by the animal's failure to revive in a specified post-exposure environment. There is no test of immediate applicability to prove that a hypothermic injury is irreversible. A possible exception is electrical asystole of the heart, which in these tests proved incompatible with recovery however abetted. On the other hand, Fairfield (13) reports spontaneous recovery of infant rats restored to air at 35° after electrical asystole for 1 hour or more.

From these data one may conclude tentatively that at this rate of cooling no guinea pig's colonic temperature can be reduced below 21°C. with impunity and that a prompt death is almost certain when the temperature falls to 17°C. Without indicating the depth of their thermocouples, Weltz, Wendt and Ruppin (14) report that in guinea pigs colonic temperatures of 15°C. for 15 minutes, 18°C. for 1 hour,

or 25 to 30°C. for about 10 hours are just compatible with survival. Such limits are lower than those suggested by the present data. When compared with results on rats (15) and on rabbits (16), the guinea pig appears to be especially susceptible to a hypothermic death. The mechanisms which led to these prompt fatalities remain obscure. Although no complete studies of ventilation and heart rate were done on

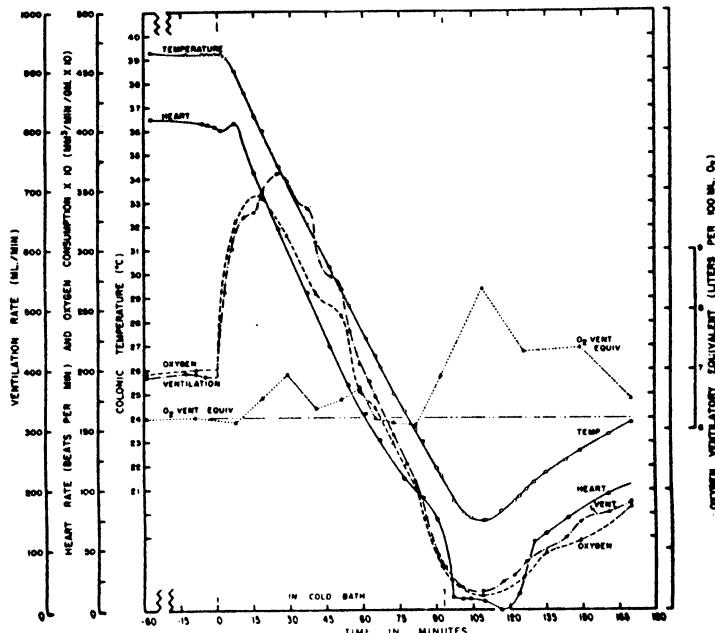


Fig. 5. OBSERVATIONS ON A SINGLE GUINEA PIG during cooling and early rewarming in room air ( $27.5^{\circ}\text{C}$ .). Jacketed animal in ice bath between times 0 and 93 (min.).

TABLE I. ANALYSIS OF SURVIVAL

CLASS	MEAN MINIMAL COLONIC TEMP. ( $\pm$ S. D.)	NO. OF ANIMALS	PER CENT OF TOTAL
A Immediate deaths.....	(18.7)	5	18
B Death after partial recovery.....	$18.7 \pm 0.8$	7	25
C Survivals.....	$19.4 \pm 1.2$	14	50
D Atypical deaths.....	19.2	2	7
TOTAL.....		28	100

these particular animals (class A) there is no reason to suspect that their responses to hypothermia were qualitatively different from those of the survivors. As previously demonstrated a failure of external respiration is not the limiting factor since relative to the oxygen uptake, the ventilation rate remained high up to the stage of terminal apnea. The latter appeared at about the same time as a precipitous drop

in heart rate, but electrical systoles generally outlasted any detectable breathing by several minutes. On the other hand, terminal deterioration is so rapid, profound and generalized that a satisfactory explanation of a hypothermic death can hardly be expected from studies of the relative times at which respiratory and cardiovascular collapses appear. Crismon (1) believes that death in cooled rats is due to cardio-circulatory failure as evidenced by an extreme drop in heart rate and in arterial blood pressure. Such an interpretation offers a tentatively acceptable explanation of the immediate deaths in the present study. In accord with previous reports, pertinent findings on post-mortem examination were few. Not infrequently, there was engorgement of the great veins, of the right heart, and sometimes of the pulmonary vascular bed, but pulmonary edema, adrenal hemorrhage and adrenal enlargement were not apparent on gross inspection.

The animals that showed a partial recovery and delayed death (class B in the table) are of particular interest. When restored to room air, their temperatures climbed slowly but steadily; their heart rates accelerated in a manner consistent at all times with their increasing colonic temperature; ventilation rates rose; spontaneous motor activity returned. Some of these animals made much faster progress in repaying their thermal debt than others, but, whatever their accomplishment, colonic temperatures tended to level off after 3 to 5 hours when shivering and struggling slackened. This left some animals with body temperatures only slightly below normal, while others were stranded at distinctly hypothermic levels. The latter were often unable to support their own weight or to take food, but this was not invariably true.

All animals were returned to individual cages. The next morning some were dead. With only two exceptions the fatalities occurred among those animals which had not succeeded in regaining colonic temperature of 30°C. within 4 hours after their immersion. By extrapolating the temperature curves of these animals who died within 10 to 15 hours of the immersion (class B), it becomes apparent that none of them succeeded in re-establishing a normal colonic temperature. Most of them ceased to warm while under observation and at colonic temperature ranging from 27.5 to 30.5°C. Among survivors (class C), either the body temperature continued to rise throughout the 4 to 5 hours of observation or it stabilized above 34°C.

It is apparent that the damage sustained during severe chilling impairs a guinea pig's ability to repay its thermal debt. In this respect, its performance is critically influenced by the environmental temperature during rewarming. Of 14 animals rewarming in still air at 25°C., 6 failed to restore a normal colonic temperature and eventually died; at 27.5°C. the environment overtaxed the restorative capacity of only one animal in 7. Can one predict at the end of the cooling which animals have received a fatal insult and will prove incapable of regenerating a normal body temperature? Within broad limits this damage cannot be estimated from the intensity of the exposure as measured by the minimal temperature reached during cooling (table 1). Electrocardiograms and studies of ventilation and of oxygen consumption during cooling do not pre-assess this damage. Indeed one animal, which eventually recovered completely and promptly, was apneic for 20 minutes shortly after cooling. Even early in the rewarming operation, oxygen consumption and ventilation rate

do not sufficiently distinguish between animals of class B and C to be of prognostic value. For predictive purposes, the vital signs studied here are inadequate.

The delayed deaths (class B) cannot be explained any more satisfactorily than they can be predicted. Probably the cooling contributed only by so impairing thermoregulation that thereafter some animals could maintain body temperature only a few degrees above that of room air. Prolonged exposure to mild degrees of hypothermia (from 28 to 31°C.) was the essential feature of these deaths. Weltz *et al.* (14) observed that with colonic temperatures held at 25 to 30°C. his guinea pigs died after about 10 hours. That a hypothermic injury may cause death after com-

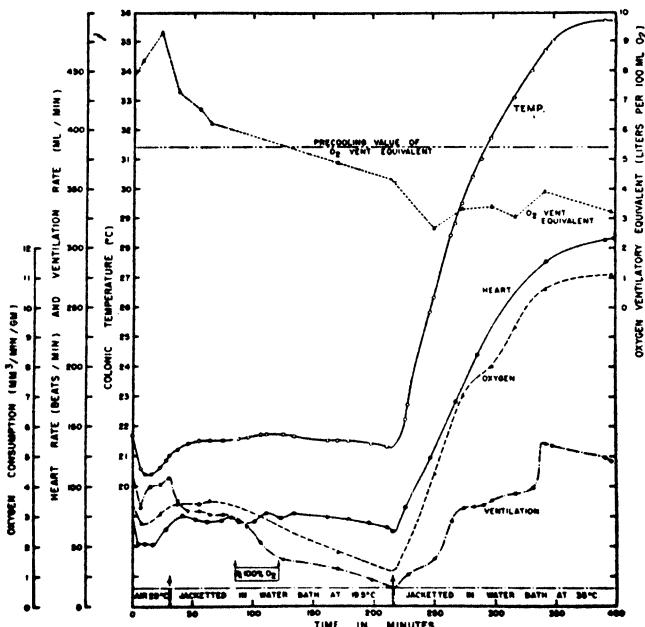


Fig. 6. OBSERVATIONS ON A JACKETED GUINEA PIG removed from ice bath at zero time, held in a cool bath for over 3 hours, and then rewarmed in a bath at 35°C. Between times 85 and 121, pure oxygen was inspired.

plete spontaneous thermal recovery has been reported occasionally but was not demonstrated in the present study.

Delayed deaths after partial recovery (class B) did not occur during the period of experimental observation. Probably the same functional disturbances occurred in more accelerated fashion when an animal was placed in a cool post-immersion environment. A thermal equilibrium was then rapidly established, and the body temperature stabilized at a value which could not be tolerated long. As illustrated in figure 6, this was accomplished by allowing a jacketed animal to rewarm in a water bath at 19.5°C. The colonic temperature promptly leveled off at about 2°C. higher. The heart rate remained constant for almost 3 hours when a downward trend began. The ventilation rate fell steadily but slowly throughout; on other occasions, it too

stabilized for over 2 hours before the inevitable decline. During such an isothermal period, alterations in heart rate were invariably preceded by a severe and sustained depression of respiration. As long as the body temperature was held constant, any downward trend in heart rate was found to signal an impending death, and this sign was thought to be more reliable prognostically than the absolute values of heart rate, ventilation rate, or oxygen consumption. It is obviously difficult to ascribe such a death to a primary cardiovascular collapse, however tenable this interpretation of the prompt fatalities in severe hypothermia (class A). As illustrated in figure 6, relative to the fall in oxygen consumption the diminution in ventilation rate was not critical. Indeed if these delayed deaths are due to cumulative anoxic damage, the primary defect does not appear to be in alveolar ventilation or in the transport of blood. Such experiments make it doubtful that any one factor is uniquely critical in all hypothermic situations.

In any case the type of deterioration illustrated in figure 6 is relatively slow and probably susceptible to appropriate supportive measures. Of the many measures suggested (2, 9, 10), only two were tested. The inhalation of pure oxygen did not modify the vital signs at any time and did not forestall their decline. The only measure of apparent benefit was vigorous artificial rewarming. In the experiment of figure 6, this was accomplished by immersing the jacketed guinea pig in a water bath held at 35°C. The restoration of temperature and of vital signs was prompt. Although the oxygen consumptions rose considerably faster than the ventilation rate, the latter was not obviously inadequate. However the ventilation rate, oxygen uptake and oxygen ventilatory equivalent at the end of the test were all appreciably lower than in animals which recovered spontaneously. It is worthy of emphasis that by all previous experience this animal was doomed by his protracted exposure and that in room air he would have survived no more than a few hours. But the low ventilatory and metabolic response seen here suggest a danger possibly inherent in rapid artificial rewarming.

#### SUMMARY

Observations were made on 30 unanesthetized mature guinea pigs during and after severe reductions of body temperature produced by immersing the jacketed animals in ice water. Measurements included colonic temperature, electrocardiograms, ventilation rate (minute respiratory volume), oxygen consumption, and carbon dioxide production. Regardless of the rate of cooling or rewarming, heart rates varied linearly with colonic temperatures between 23 and 35°C., falling 17.5 beats per minute for each decrement of one Centigrade degree. Below 23°C. the drop was erratic and often precipitous. Sinus and nodal arrhythmias, premature beats of auricular and ventricular origins, auriculoventricular blocks, and reversible ventricular fibrillation were all noted.

The early phases of cooling were characterized by increased struggling, hyperpnea, enhanced metabolism, and cardiac slowing. When cooling at 0.2 to 0.3 degrees per minute, maximal ventilation occurred at 35°C. (33° to 37°) and averaged 170 per cent of the pre-cooling rate. At this point maximal rates of oxygen consumption also amounted to 170 per cent of the pre-cooling level or 250 per cent of the

estimated basal. Below 33°C., ventilation and oxygen consumption diminished progressively with temperature, dropping below pre-cooling rates between 26 and 29°C. and approaching zero slightly below 20°C. Relative to the oxygen uptake, however, the ventilation rate (expressed as the oxygen ventilatory equivalent) remained high to the stage of terminal apnea. Therefore, the progressive fall in oxygen consumption cannot be ascribed to failure of external respiration.

Some animals survived briefly at 17.5°C., others succumbing at as high as 21°C. This large variation in lethal temperature is unexplained. The mechanisms of a hypothermic death remain obscure, but these data suggest that in severe chilling critical circulatory inadequacy often precedes respiratory failure.

Guinea pigs which did not die promptly in deep hypothermia were allowed to rewarm in room air. During rewarming ventilatory and metabolic rates varied widely among the tests, and the temperature of some animals rose much more slowly than that of others. It is postulated that damage sustained during severe chilling impairs the capacity to repay a thermal debt. It was not possible to predict final rates of recovery from vital signs during cooling or even during the early phases of rewarming. Beyond 4 to 6 hours after the immersion, relatively little progress was made in raising the body temperature, leaving some animals stranded at distinctly hypothermic levels (about 30°C.). The latter died within 10 to 15 hours of the immersion. Slight departures of the heart rate from predictions based on the colonic temperature appeared to signal such impending deaths. Whatever the derangements responsible for these delayed fatalities, it seems probable that they represent controls different from those operating at the lethal temperature during cooling.

The author gratefully acknowledges the sympathetic cooperation and guidance of Dr. E. F. Adolph. Dr. A. B. Otis generously furnished the respiratory valves and the design of the respiratory mask used here. Miss M. Suskind kindly performed the gas analyses.

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# ANALGESIA AND ANESTHESIA INDUCED BY EPINEPHRINE

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**E**ARLIER investigations (1) have shown that the intracisternal (i.c.) injection of epinephrine (epn) in large doses induces analgesia, drowsiness and sometimes sleep. These observations are in accord with the findings of Ivy (2) and co-workers, namely, that epn injected into the carotid arteries of dogs produces analgesia. On the basis of these observations, experiments were performed to evaluate the intrathecal injection of epn for surgical anesthesia.

## METHODS

Twenty-six experiments were made on dogs. In the first 2 experiments, epn solution (Winthrop) was employed, but in the remainder, epn-base-powder (Parke-Davis) was used to eliminate the preservatives, sodium bisulfite and chloro-butanol, which perhaps might contribute to the anesthesia. The epn-powder was dissolved in 1 or 2 cc. sterile, distilled water and slightly acidified with diluted HCl. Two or three cc. of cerebrospinal fluid (CSF) were removed and discarded. The epn-solution, placed in a syringe, was diluted by directly and repeatedly withdrawn CSF (barbotage). Due to the alkalinity of the CSF, the pH of this mixture, as seen in repeated tests, was approximately 7.4. The diluted epn-solution was then injected i.c. The volume injected was always adjusted to approximate the volume of CSF originally withdrawn. At the end of the injection, a small amount of CSF was again drawn into the syringe to demonstrate that the entire injection was intracisternally. When the CSF was blood-tinged no experiment was performed.

In 6 experiments, epn alone ( $\frac{1}{2}$ , or 1 mg/kg.) was injected i.c. without any preliminary sedatives. In another group of experiments, small amounts of nembutal (20 mg/kg.) were injected intraperitoneally (i.p.) as basal anesthesia (b.a.). This was done in order to decrease the amount of epn necessary to produce anesthesia and to make the i.c. injection less difficult procedure. Several hours after the nembutal injection, when the dogs were awake, epn ( $\frac{1}{2}$  mg/kg.) was injected i.c.

In 3 other experiments, ephedrine sulfate (in the amount of 5 mg. or 15 mg/kg.) was given i.c. with preliminary b.a. In a few experiments, NaCl-solutions (0.9% and 15%) were injected i.c., also with previous b.a. and in one experiment procaine-HCl (in the amount of 6 mg/kg.) was administered i.c. without preliminary sedation.

In most of these experiments, the blood pressure (by way of a cannula from the femoral artery), the respiration (by means of a pneumograph) and the electrocardiogram (ECG, by a directly writing apparatus, Sanborn,) were recorded. In a few experiments, electroencephalographic records (EEG, by means of a Grass-amplifier and inkwriter) were taken.

In another group of experiments (including 11 guinea pigs and 1 dog), epn in different concentrations was injected intracutaneously (into the outer side of the right hind leg): 1 cc. of 1:30000 (9 guinea pigs and one dog); 1 cc. of 1:40000 (2 guinea pigs).

For control, 1 cc. of physiological NaCl was injected intracutaneously into the corresponding area of the opposite hind leg.

## RESULTS

**Action of i.c. epn.** When epn alone ( $\frac{1}{2}$  or 1 mg/kg.) had been injected the dogs became quiet within 10 to 15 minutes (following a brief period of excitement) and 30

Received for publication January 17, 1949.

minutes after injection they were asleep. Sleep occurred earlier when the head of the dog was maintained in a lower position than its body. The sleep lasted for 1 to 2 hours. The respirations were deep and regular all this time. About  $\frac{1}{2}$  hour after the onset of sleep, pinching, cutting or suturing the skin elicited no signs of pain from the dogs (i.e. restless movements of the legs, growling or whining). To perform a laparotomy an additional amount of epn ( $\frac{1}{2}$  mg/kg.) had to be injected i.c. about  $\frac{1}{2}$  hour after the previous injection. Following this, a complete laparotomy did not result in any evidence of pain. Specifically, the abdominal muscles could be cut, the

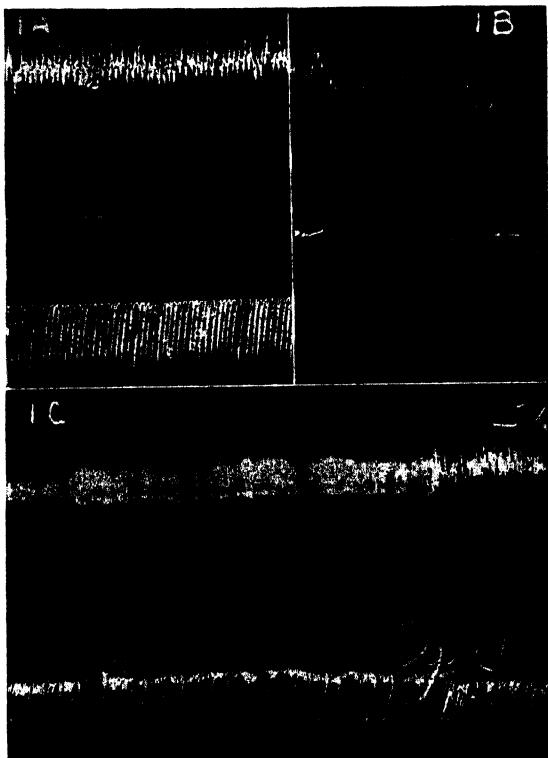


Fig. 1. DOG 11 KG. *Upper tracing*: blood pressure; *lower tracing*: respiration. (A) before injection; (B) after i.c. injection of 5 mg. epinephrine (powder). (C) DOG 15 KG.; *upper tracing*: blood pressure, *lower tracing*: respiration during a laparotomy after i.c. injection of 15 mg. epinephrine (powder).

peritoneum incised and traction could be exerted on the peritoneum and mesentery. The degree of relaxation of the abdominal muscles was complete.

Usually 4 hours after the nembutal injection, in the second group of the epn experiments, the animals were fully awake so that they struggled to free themselves from the animal board and gave distinct signs of pain on pinching the skin. Epn ( $\frac{1}{2}$  mg/kg.) was then injected i.c. After a short period of excitation, the dogs fell asleep within 10 minutes and remained in sleep for 3 to 4 hours. Again the onset of sleep was hastened by lowering the dog's head. Approximately, 30 minutes after the injection, complete surgical anesthesia occurred. In 7 such experiments, laparotomies were performed without any evidence of pain on the part of the dog.

The blood pressure, observed during the entire experiment, even during a laparotomy, remained at the normal level (fig. 1*A*, *B*, *C*). The ECG also remained without any change (fig. 2*A*, *B*). In contrast, the ECG of the same dog showed many ventricular extrasystoles after intravenous injection of 1 mg epn (fig. 2*C*, *D*) and the typical increase in blood pressure occurred.

Electroencephalographic studies revealed no significant change after i.c. epn in the doses which produced anesthesia. Only after the administration of extremely large doses ( $2\frac{1}{2}$  to 3 mg/kg.) were the EEG-waves greatly depressed in voltage. Later the electrical activity of the brain ceased. At the same time, the ECG showed ventricular fibrillation and the dog died. The injection of these tremendous amounts of epn involved changes which could be attributed to the effects of hypertonicity, as

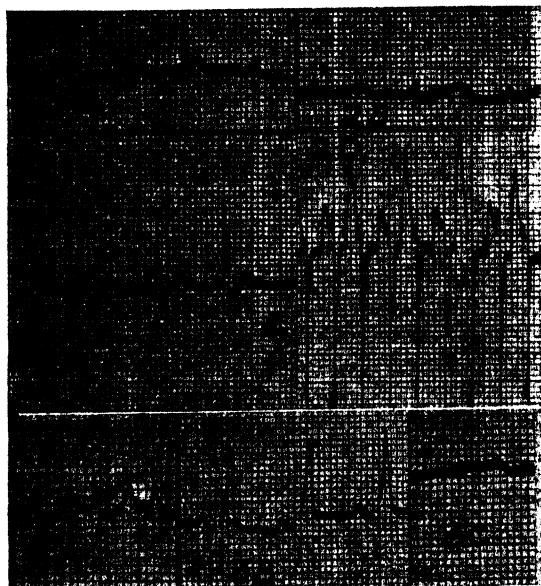


Fig. 2. DOG 14 KG. ECG: (A) before injection; (B) after i.c. injection of 8 mg. epinephrine (powder). Same dog ECG: (C) before injection; (D) after i.v. injection of 1 mg. epinephrine (powder).

Fig. 3. DOG 10 KG. (A) ECG before injection; (B) ECG after i.c. injection of ephedrine sulfate 15 mg/kg. (note high anoxic T-waves); (C) ECG 10 minutes after i.c. injection of ephedrine sulfate 15 mg/kg. (note ventricular fibrillation).

will be explained later. After i.c. epn, the respiration was not depressed; on the contrary it was usually stimulated, mainly by increase in amplitude (fig. 1*A*, *B*).

It should be mentioned that sometimes, especially in the experiments without nembutal, a transitory rigidity of the forelegs and of the neck appeared shortly after the i.c. injection of epn. This stiffness disappeared completely on the following day.

All the dogs in the above-mentioned groups except two (into which excessively large doses of epn ( $2\frac{1}{2}$ -3 mg/kg.) had been injected purposely) were in good condition the day following the i.c. injection, even though a laparotomy had been performed. They remained so during the time of observation (2-4 months). No after-effects were seen during this time, even though, in some cases, 3 or 4 i.c. injections of epn, at various intervals, had been made into the same dogs.

*Action of i.c. Injection of Ephedrine Sulfate.* Following i.c. ephedrine (5 mg/kg.)

no analgesia and no tendency to sleep could be detected (in 2 experiments). The blood pressure rose from 130 to 200 mm. Hg and remained at this high level for the time of observation ( $1\frac{1}{2}$  hours). The ECG showed many ventricular extrasystoles and later high anoxic T-waves. After about 40 minutes normal ECG-waves reappeared. In one experiment, after i.c. ephedrine 15 mg/kg., likewise no evidence of analgesia or sleep could be observed. The blood pressure rose from 130 to 210 mm. Hg and 20 minutes later fell suddenly to zero (fig. 3). The ECG revealed severe changes, many ventricular extrasystoles, high anoxic T-waves and finally ventricular fibrillation with resulting death of the dog (fig. 3A, B, C).

*Action of i.c. Procaine-HCl.* In one experiment, following procaine HCl (6 mg/kg.) pronounced dyspnea and severe cyanosis appeared almost immediately. No anesthesia to pricking or pinching was found. No sleep occurred. A transient paresis of the hindlegs lasting one hour was observed (the dog had been placed in Fowler's position). Within  $1\frac{1}{2}$  hours the dyspnea and cyanosis disappeared.

*Action of the Intracutaneous Injection of Epn.* All the guinea pigs and the one dog showed analgesia to pricking, pinching and cutting 10 to 15 minutes following the intracutaneous injections of 1:30,000-1:40,000 epn. This analgesia lasted from 24 to 48 hours. No necrosis was seen at the site of the injection. The sites of saline-injection revealed no analgesia or only temporary hypalgesia for a few minutes.

#### DISCUSSION

The fact that general anesthesia and sleep can be induced by i.c. epn indicates that this substance exerts an effect on some center or centers in the brain (perhaps hypothalamus or brain stem). The question may arise whether the observed phenomena might be attributed to vasoconstriction following i.c. epn administration. Such an assumption does not hold. Fog (3) Forbes (4) and co-workers have shown that the direct application of epn to the pia mater produces no constriction of the arterioles and only slight short-lasting constriction of the large arteries. In our experiments, however, sleep and analgesia lasting several hours were produced.

There is other evidence of the effect of epn on the central nervous system without concomitant vasomotor action. In our previous experiments (1) it was demonstrated that epn injected intrathecally causes a rapid, high sustained rise in blood sugar without changes in the blood pressure and the ECG. The analgesic effect of intracarotid injection of epn in dogs, shown by Ivy (2) and co-workers, suggests a direct action of epn upon pain perceiving centers. Further, the recently reported thermogenetic effect of epn on the brain (Pick and Feitelberg) (5) reveals a central action of epn without accompanying blood pressure effect.

From our present experiments, we conclude that epn has central analgesic and anesthetic properties. Moreover, the long-lasting local anesthesia after intracutaneous administration of epn suggests that epn has a direct effect on peripheral nerves independent of vasomotor action and is probably capable of blocking nerve conduction.

In this connection it might be mentioned that recently reported experiments seem to demonstrate that there exists a relationship between morphine-analgesia and epn. The analgesic response to morphine of rats (6) and dogs (7) was markedly reduced

after removal of the adrenal medulla. Other earlier experiments have shown that morphine causes the release of epn from the adrenal medulla (8).

With regard to the question, whether the i.c. injection of the above-mentioned large doses involves some danger to the cardiovascular system, there might be said the following. It was demonstrated in earlier experiments that epn injected intrathecally does not raise the blood pressure (1/10/11). The same was seen in these experiments during which much larger doses were employed.

In order to avoid a rise in blood pressure, it is necessary to take three precautions: 1) Epn must never be injected i.c. when the withdrawn cerebrospinal fluid is bloody or blood-tinged; otherwise epn can enter the general circulation. 2) I.c. injection of epn should be made only after removal of a volume of CSF equal to the volume of fluid to be injected. 3) A hypertonic solution of epn should never be injected. This can be avoided by dilution of the epn-solution in the syringe through repeated mixing

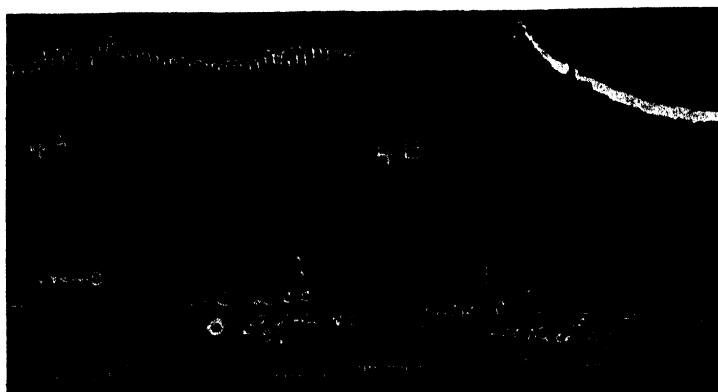


Fig. 4. DOG 11 KG. Nembutal anesthesia. *Upper tracing:* blood pressure; *lower tracing:* respiration. (A) after i.c. injection of 2 cc 0.9% saline pH 7.4; (B) after i.c. injection of 2 cc NaCl 15%.

with CSF (barbotage). Further, a large amount of epn should not be injected i.c. at one time, but in small fractions at proper intervals. When these precautions are taken the ECG does not change either. In this connection, it should be pointed out that a hypertonic NaCl-solution itself, injected i.c. can provoke a pronounced rise in blood pressure and severe changes in the ECG (fig. 4).

Of great importance is the fact that the respiration is not depressed after i.c. epn, but on the contrary is stimulated (probably by direct stimulation of the respiratory center). Crystalline epinephrine base seems to be more effective than other epinephrine preparations.

Another sympathomimetic drug, ephedrine sulfate, i.c. does not produce analgesia nor sleep in the dog. Nor has procaine-HCl an analgesic effect after i.c. injection in contrast to its well-known action after lumbar injection. There exists a tendency among anesthesiologists to use epn (11, 12) or ephedrine (13, 14) as an adjunct in procaine spinal anesthesia. Our experiments suggest that epn might aid in this analgesia not by ischemia, but synergistically as an analgesic. Caution

should be exercised in the intrathecal injection of ephedrine as an adjunct in spinal anesthesia.

#### SUMMARY

Intracisternal injection of epinephrine alone ( $\frac{1}{2}$ -1 mg/kg.) produces surgical anesthesia in dogs. An additional amount of epn ( $\frac{1}{2}$  mg/kg.) is necessary to accomplish complete surgical anesthesia for laparotomy. When small amounts of nembutal are injected intraperitoneally several hours prior to i.c. epn, a smaller amount of epn ( $\frac{1}{2}$  mg/kg.) is adequate for complete surgical anesthesia. The blood pressure, ECG and the EEG remain normal after i.c. epn in the mentioned doses. The respiration is not depressed but stimulated (mainly in amplitude). No after effects are observed. Excessively great doses of i.c. epn (about  $2\frac{1}{2}$ -3 mg/kg.) are toxic. The possibility of extending these investigations for use in operation on man is discussed.

The i.c. injection of ephedrine sulfate or procaine-HCl does not produce analgesia or sleep in the dog.

The authors thank Mr. G. Warner for his technical assistance.

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# MEASUREMENT OF EXPERIMENTALLY INDUCED BRAIN SWELLING AND SHRINKAGE<sup>1</sup>

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**I**N STUDIES of the mechanisms of cerebral edema and swelling a simple method for estimating the extent of changes in brain volume in experimental animals is needed. In the present paper it is shown that determinations of dry weight provide a simple method for estimating variations in brain tissue volume, and some observations on swelling and shrinkage are described.

## MEASUREMENT OF CHANGE IN BRAIN VOLUME

White *et al.* (1, 2) have measured changes in brain volume in cats by careful determinations of the volume of the brain and of the cranial cavity. The normal difference between these two volumes is about 10 per cent and variation from this figure represents swelling or shrinkage of the brain. This method is difficult, and could hardly be applied after craniotomy. Assuming that changes in brain volume are due to changes in water content, and that the percentage of dry matter in the brains of normal animals is constant, then a difference from normal in the percentage dry weight of the brain of an experimental animal must be a measure of a change in volume. The swelling or shrinkage may be simply calculated without any need to determine the actual volume of the brain.

If W and D are respectively the fresh and dry weight of the brain of a normal animal and P is the percentage dry weight, then  $W = D \times \frac{100}{P}$ . If, as a result of treatment, the weight and percentage dry weight change to  $W_1$  and  $P_1$ , then  $W_1 = D \times \frac{100}{P_1}$ . Whence  $W_1 = \frac{P}{P_1} W$ . The swelling or percentage change in weight (or volume, since the tissue density is about unity) is given by swelling percentage =  $\frac{W_1 - W}{W} \times 100 = \frac{P - P_1}{P_1} \times 100$ .<sup>2</sup> It should be noted that presentation of results

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Received for publication January 5, 1949.

<sup>1</sup> This work was aided by grants from the Associate Committee for Army Medical Research of the National Research Council of Canada.

<sup>2</sup> If the swelling or shrinkage is caused by the absorption or loss of fluid which itself contains some solid matter, then a larger change in volume would correspond to a given change in percentage dry weight. If p is the percentage dry weight in the fluid absorbed or lost, it can be shown that

$$W_1 = \frac{P - p}{P_1 - p} W \text{ and percent swelling} = \frac{P - P_1}{P_1 - p} \times 100.$$

in terms of percentage moisture, as is commonly done, rather than dry weight, tends to give a misleading impression. If the normal moisture content is 80 per cent, a change to 81 per cent indicates a 5 per cent increase in weight since  $W_1 = \frac{20}{19} W = 105W$ .

#### DETERMINATION OF DRY WEIGHT

Rabbits were anesthetized with Nembutal and decapitated. The roof of the skull was removed and the cerebrum was removed from the skull by cutting through the mid-brain along the bony edges of the incisura of the tentorium. This method was adhered to carefully so that the parts of the brain treated, particularly with respect to the relative proportions of grey and white matter, were always the same. The cerebrum was halved midsagittally and the determination was carried out on one half, or on both halves separately as duplicates. All free fluid was carefully wiped out of the ventricles with filter paper. The whole hemisphere was then pushed into a tared weighing bottle with a helmet-type cover and containing a short sealed glass tube with a mace-like head (figure 1). This process was done rapidly, or in a humid chamber, since loss of moisture by evaporation could be appreciable. After determining the fresh weight of the hemisphere, 2 ml. of acetone were run into the bottle and the tissue was carefully reduced to a suspension by mashing with the 'mace'. The acetone was then evaporated away by directing a current of filtered air into the bottle. When the tissue could be spread as a paste around the sides of the bottle, it was placed in an oven at about 108° for 24 hours or more, cooled in a desiccator and weighed. Some care was necessary to insure that foaming of residual acetone did not cause loss of material when the temperature was first raised. After 24 hours, further loss in weight was negligible. Without the acetone treatment, complete drying took much longer. There was never any increase in weight, as has been reported for some fatty tissues on prolonged heating, following this method of acetone treatment.

The agreement between values obtained for the percentage dry weight of left and right hemispheres from the same animal was good. In 25 such pairs of determinations the widest difference was 0.3; usually the difference was much less. However, rather wide variations between individual animals were found (table 1), which could not be correlated with depth or period of anesthesia, weight, or method of killing (bleeding, decapitation, or constriction of the neck).

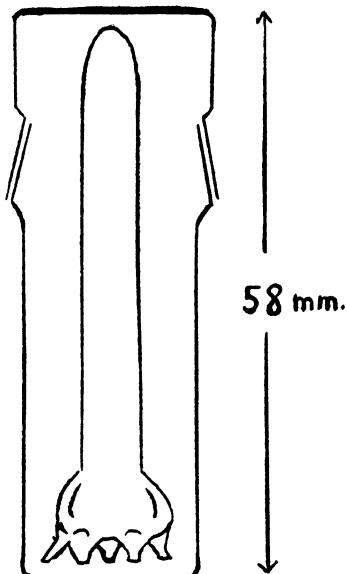


Fig. 1. WEIGHING BOTTLE WITH MACE for determining the dry weight of brain. Larger bottles could be used for brains of larger animals.

The average value for normal animals was 21.16 per cent<sup>3</sup> with a standard deviation of the distribution equal to 0.5. In view of the range of variability of the normal animals, values for brains of individual experimental animals between 20.2 and 22.1 per cent cannot be regarded as significantly different from normal. That is to say, a change of less than 4 per cent in brain volume, calculated on the basis of the average normal percentage dry weight, cannot be reliably ascribed to the treatment of the animal.

While this work was in progress a paper by Windle *et al.* (3) appeared in which a similar method for determining brain dry weight was described. This method appears to be extremely accurate but somewhat more cumbersome than the present method. The tables of Windle *et al.* show that the dry weight content of normal guinea pig brains varied between 20.5 and 21.7 per cent, a range of variability approaching that found by us for rabbits. Windle *et al.* found a statistically significant increase of 0.5 per cent in the mean moisture content of brains from concussed animals and an increase of 0.7 per cent with animals which had been water-loggued by stomach tube. It was concluded that edema following concussion is significant but slight. Calculation shows that an 0.5 per cent increase indicates about 2.5 per cent swelling which is appreciable if the available space is only 10 per cent. In earlier work Pilcher (4, 5) attempted to detect changes of brain water in traumatized dog brains, by determinations of dry weight in various parts of the brain. His tables also show great variability; values were reported for the dry weight content of cerebral grey matter between 20.1 and 21.7 per cent in 5 normal animals and between 18.7 and 22.5 per cent in unexposed sides of unilaterally exposed brains. Average figures indicated only slight, though definite, increases in moisture content following trauma with the skull intact and none with the skull exposed.

The variability of the percentage dry weight of normal brain seriously limits the precision of determinations of swelling or shrinkage by the dry weight method. Donaldson (6, 7) in extensive studies on rats, has shown that the moisture content of the brain is affected in a regular manner by the age and size of the animal and by the size of its brain. Even with animals from the same age group there is rather wide variability (s.d. 0.2 to 0.5) but this variability is considerably less among litter mates (s.d. 0.13).

#### EXPERIMENTALLY INDUCED CHANGES IN BRAIN VOLUME

Weed *et al.* (8-12) showed that considerable changes in brain bulk and spinal fluid pressure could be produced in cats by intravenous injections of hypo- and hypertonic fluids. Similar procedures have been used here. Portions of the skulls of rabbits, anesthetized with Nembutal, were removed and the dura reflected to expose the brain. Either 0.1 per cent glucose solution (hypotonic fluid) or 25 per cent glucose (hypertonic fluid) was infused into the femoral vein, at a rate of about 2 ml. per minute, usually for 60 to 75 minutes. Sometimes the fluid was infused into the

<sup>3</sup> It should be noted that the value, 21.16 per cent, for the average dry weight content has no absolute significance since it represents only a particular mixture of grey and white matter from a variety of brain regions. It is of value only for comparison with similar brain samples from different animals.

internal carotid artery but the results were about the same. The percentage dry weight of the brains of animals thus treated and the change in brain volume calculated therefrom are shown in table 2.

Hypotonic infusion caused definite swelling of the brain tissue. Volume changes were found between 6 and 15.5 per cent, calculated on the basis of average normal dry weight. Hypertonic infusion caused very marked shrinkage, up to 31 per cent in the case of one animal infused for 3 hours. The shrinkage of the brain relative to the cranium was very obvious in all cases of hypertonic infusion and noticeable within 20 minutes of starting the infusion. Chemical determinations on the brain of one of these animals showed that excess of glucose or lactate in the brain could not account for an appreciable fraction of the increased percentage dry weight.

TABLE I. VARIATION IN DRY WEIGHT CONTENT OF NORMAL RABBIT BRAINS

% dry weight .....	20.1	20.3-20.5	20.6-20.8	20.9-21.1	21.2-21.4	21.5-21.7	21.8-22.1	22.3
No. of animals.....	I	0	4	7	6	2	2	I

TABLE 2. EFFECTS OF INTRAVENOUS INFUSION OF HYPO- AND HYPERTONIC FLUIDS ON PERCENTAGE DRY WEIGHT OF BRAIN

HYPOTONIC INFUSION		HYPERTONIC INFUSION		HYPOTONIC INFUSION		HYPERTONIC INFUSION	
Per cent dry weight	Per cent <sup>1</sup> swelling	Per cent dry weight	Per cent <sup>1</sup> swelling	Per cent dry weight	Per cent swelling	Per cent dry weight	Per cent swelling
<i>Brain exposed</i>							
19.1	+11	23.6	-10.5	19.85	+6.5	21.85	-3
20.0	+6	24.7	-14.5	21.05	+0.5	22.4	-5.5
18.5	+14.5	26.7	-21	21.5	-1.5	23.4	-9.5
18.3	+15.5	24.6	-14	20.35	+4	23.4	-9.5
19.7	+7.5	30.8 <sup>2</sup>	-31	19.5	+8.5		
19.5	+8.5			21.35	-1		
19.2	+10						
<i>Skull intact</i>							

<sup>1</sup> Calculated on the basis of 21.16% dry weight for normal brain. Values less than  $\pm 4$  are probably within the normal range.

<sup>2</sup> Infused 3 hours, till death of animal.

The marked changes in brain volume described above all occurred with the brain exposed. When infusions, hypo- or hypertonic, were carried out with the skull intact the effects on the brain volume were less marked (table 2). Evidently mechanical-hydrostatic effects in the closed system counteract osmotic effects. Reid (13) observed less marked histologic effects with cats following water infusion with the skulls intact. Weed and McKibben (12), however, found histological changes following hypo- and hypertonic injections only when the skull was intact.

It may be mentioned that the amount of 25 per cent glucose solution administered during 60 to 75 minutes would correspond to 2.5 to 4.0 liters to a 70-kg. man. Yet not one of the animals so treated, with the brain exposed or the skull intact, showed any obvious signs of distress. Diuresis was prolific, but there was no hemo-

concentration; usually there was slight hemodilution as judged by hematocrit and hemoglobin determinations before and after infusion. Only the animal treated for 3 hours died. With hypotonic infusion there was no diuresis and several of the animals died.

Histological observations were made by Dr. Karl Stern. After hypotonic infusion there was swelling of many cortical nerve cells and enlargement of intercellular and perivascular spaces. The nerve cell change was most characteristic in silver stain (Bielchowsky). There was an unstained halo around the slightly enlarged nucleus and the argentophile substance was 'squeezed' in fragments to the periphery of the cell. Reid (13) found less effect on nerve cells, but constant marked swelling of oligodendroglia and no significant change in other cellular elements. The picture in areas of edema surrounding a brain tumor in man was quite different from that seen in his animals after experimental edema. The brains which had been dehydrated and shrunken by hypertonic infusion in our experiments showed, in silver stain, a peculiar nerve cell picture not unlike the one encountered in the early stages of 'senile' changes in man. The intracellular fiber strands were markedly argentophile and showed clumping and coarseness. The intercellular and perivascular spaces showed a normal picture. Details of these observations on shrunken brains are discussed in another publication (14).

These experiments and histological observations indicate that the dry-weight method does detect and roughly measure cerebral edema and dehydration. Edema from causes other than infusion of hypotonic fluid can apparently also be detected. In a series of 5 experiments no infusion was administered but, after unilaterally exposing the brain for periods up to two hours, muscle and scalp were sutured over the skull defect and the animals kept alive for two days. With 3 of these animals, dry-weight determinations indicated swelling of 3, 5.5 and 6.5 per cent. With two animals, in which the exposure was very brief, no swelling was measurable. In no case was there measurable swelling of the unexposed hemisphere.

In 9 experiments no fluid was infused into the venous system but the brain was exposed for about two hours during which it was either left dry or its surface was irrigated continuously with normal saline, Ringer's, hyper- or hypotonic glucose solution or plain water. The animal was then decapitated and the brain dry weight determined. Results showed no correlation with the type of irrigation fluid used and all were within the normal range. But the average of the series, 21.6 (s.d. 0.6) corresponding to a 2 per cent shrinkage, was significantly different from the normal average and suggests a slight tendency to dehydration of the brain during exposure.

Brains removed from animals which had been left with skull defects for two days, and from some of the animals infused with hypotonic fluid, showed elevated areas moulded to the outline of the skull defect. These are presumably regions of local edema probably developing as a result of interference with circulation by the pressure of the herniating brain against the skull defect. The excess of moisture in these small zones would be too small to affect measurements on the whole brain. Attempts to measure local edema by dry weight determinations on small local areas of the rabbit brain were defeated by too great variability in tissue samples from normal brains.

Other methods for chemical evaluation of cerebral edema have been tested in a preliminary way. These depended upon the possibility that the edematous process involved a change in the amount of brain water which is free to dissolve various substances present in the blood. For instance the chloride space of the brain is about 40 per cent of the total tissue volume instead of 80 per cent which would be expected if all the tissue water were free to dissolve chloride. If the extra fluid entering the tissue in edema contained the same concentration of chloride as the plasma, the chloride content of the brain should increase to a relatively greater extent than does the water content. It can be shown that an increase of 5 per cent in the fluid content of the brain should under these circumstances change the chloride space to 43 per cent, making a 7.5 per cent increase in chloride space. The thiocyanate space is only about 15 per cent and a 5 per cent increase in brain volume made up entirely of water, free to dissolve thiocyanate, would increase the thiocyanate space to 19.7 per cent, which is a relative increase of 31 per cent. With inulin, which normally enters the brain fluid only slightly, the relative increase in edema might be very high. All these possibilities have been tested by determinations of the substances in question in the plasma and in the brain, correcting the amount in the brain for the portion accounted for by blood remaining in the brain. Sodium thiocyanate was administered intravenously or potassium thiocyanate by stomach tube at least 2 hours or 8 hours, respectively, before killing the animal; inulin was given intravenously about 35 minutes before sacrificing the animal. The chloride space of normal brains was found to be reasonably constant, values of 37.1 to 39.6 being obtained, the thiocyanate space seemed to vary widely and no inulin at all appeared to enter the normal brain. All these methods involved accurate determinations of the materials in the blood and in the brain and of the blood content of the brain. The thiocyanate and inulin methods involved interference with the animal, while the chloride method offered little increase in sensitivity. These methods were therefore not pursued when the simple dry-weight method was found to be reasonably satisfactory. But this type of experiment might give valuable information concerning the mechanism of the development of edema.

#### BRAIN SWELLING WITHOUT TRUE EDEMA

A rapidly developing swelling of the brain, with tendency to herniation through the skull opening, is an occasional, but disconcerting, experience of anyone who has done extensive surgery of the brain in man or in experimental animals. Pilcher (4) observed marked bulging of the brain in one out of 6 dogs following exposure of the cortex without trauma, and in 4 out of 5 when trauma to the head preceded exposure. He was unable to show, however, that this was accompanied by a significant increase in water content of the brain in these animals. He concluded (5), "It seems probable that other factors, such as cerebrospinal fluid volume and intracranial blood volume are of greater importance than cerebral edema in producing the increased intracranial pressure which follows trauma to the head."

Prados *et al.* (15) in studies on the effects of exposure on cat brains, reported swelling usually observed about two hours after exposure. "The degree of swelling varied a great deal from one experiment to the other, and it depended on some factor the nature of which we are not yet able to determine." In our experiments on rabbits, marked herniation of the brain occurred in 4 out of 18 cases after simple exposure of the cortex for an hour or more without obvious trauma. We were unable to determine the conditions which provoked this swelling, since it did not seem related to the type of irrigating fluid being used, nor did it depend upon whether the cortical surface was kept moist or allowed to dry. The herniation subsided upon severing the neck of the animal. Brain dry-weight determinations in two such cases where herniation had occurred indicated that no swelling of the tissue due to increased fluid content had occurred.

These observations serve to emphasize again the importance of a type of swelling which can occur independent of actual change in brain tissue volume. It presumably results from blood vessel dilatation or dilatation of ventricles and cisterns. The mechanism is one of 'inflation' rather than edema. It is commonly seen in very acute form when the animal struggles and cerebral vessels become engorged. It can be readily imitated by increasing the blood volume by rapid intravenous injections or by increasing the spinal fluid volume by intracisternal injections. It may result from changes in blood volume or pressure or from increased spinal fluid volume produced by increased rate of secretion, decreased rate of absorption, or displacement of fluid from the spinal canal into the ventricles. Such events may occur as a reaction to chemical products of trauma or as a result of nervous reflex reaction to certain cerebral stimuli. Obrador and Pi-Suner (16) have described sudden inflation of exposed dog brain on the production of lesions near the fourth ventricle. The mechanism should be susceptible to partial analysis by determinations of blood in the cranium following sudden constriction of the neck by the method of White *et al.* (2). Results of some preliminary trials did not indicate that excess blood could account for swelling and herniation observed.

The observations of inflation of the brain have all been made upon exposed brains. When the skull is intact, factors which tend to produce inflation may still operate and it seems probable that some cases of raised intracranial pressure may be due partly to these factors and not entirely to true tissue edema or space-occupying lesion.

In the experiments described earlier, on infusion of hypotonic fluid into rabbits with exposed brains and on rabbits which had been kept for two days with a large skull defect, marked herniation of the brain occurred. This herniation usually subsided considerably on decapitation even though dry-weight determinations showed that there was an appreciable true increase in brain volume. In these animals, therefore, both true edema and inflation were induced.

#### SUMMARY

The approximate extent of swelling or shrinkage of brains of experimental animals can be readily calculated from the dry weight of the brain without knowledge of the actual brain volume. A simple method for determining the dry weight is described.

The moisture content of normal rabbit brain varies considerably. Changes in moisture content well beyond the range of normal variability were produced by intravenous infusions of hypo- or hypertonic solutions. Such changes were more marked when the brain was exposed than when the skull was intact. The difference between true edema, or swelling due to excess water in the brain, and an apparent increase in volume due to hydrostatic effects is discussed.

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# PULMONARY LESIONS IN GUINEA PIGS WITH INCREASED INTRACRANIAL PRESSURE, AND THE EFFECT OF BILATERAL CERVICAL VAGOTOMY<sup>1</sup>

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**E**DEMA and congestion of the lungs are frequently encountered at necropsy in head injury cases. Moutier (1) noted the frequent occurrence of fatal acute pulmonary edema within 24 hours after local cerebral trauma associated with battle injury. He attributed these deaths to pathological hyperactivity of the suprarenal glands. Antonini and Biancalani (2) reported lung edema in 41 per cent of 82 cases of head injury surviving from several minutes to several days. Astuni (3) presented a similar series of 197 autopsied cases in which 29 per cent displayed pulmonary edema. Weisman (4) collected data on the weights of the lungs in 686 cases of traumatic and spontaneous intracranial hemorrhage. In approximately two-thirds of the cases the combined weight of the lungs was greater than 900 gm., while in a control group only 2 per cent showed lungs of this size. The increased weight was due chiefly to edema and congestion, and partly to pneumonia. Pulmonary edema and congestion developed in most cases of fatal intracranial hemorrhage within 30 minutes to one hour after the injury.

Several investigators have studied experimental pulmonary edema following nervous system damage. In 1874, Nothnagel (5) reported the death of a rabbit from pulmonary congestion after probing the brain at unspecified points. Benassi (6) introduced fluid into the cranium through a trephine opening in order to obtain hypertensive coma in rabbits and dogs. In some (numbers not stated) cases, rales were heard on auscultation of the lungs, but frothy fluid was not observed in the respiratory tree. Post-mortem examination of the lungs revealed subpleural ecchymoses, emphysema, intense vascular congestion, and sometimes partial edema.

Farber (7, 8) produced fatal lung edema in guinea pigs with bilateral cervical vagotomy and attributed this edema to disturbances of the vasomotor control of the pulmonary vessels. He maintained the animals on artificial positive pressure respiration following tracheal cannulation, (insufflation pressure not stated). Various investigators (9, 10) have presented contradictory evidence, and recently Sussman *et al.* (11) stated, "There is no evidence that vagotomy exerts an influence on the pulmonary vascular system favoring edema or hemorrhage."

Sussman found massive lung edema within six hours in either intact or vagotomized guinea pigs maintained on artificial respiration at 20 mm. Hg insufflation pressure in contrast to the absence of lung edema in such animals with an insufflation pressure of 6 mm. Hg. Luisada and Sarnoff (12) employed massive, rapid venous infusion in dogs simultaneously with vagal stimulation and concluded "electrical stimulation of either the cardiac end of the cut vagi or the intact nerve favors pulmonary edema by causing extreme bradycardia." Recently, Surtshin, Katz, and Rodbard (13) have questioned whether in previous attempts to produce pulmonary edema by elevated intracranial pressure the effects were due to genuine edema or were artefacts resulting from the aspiration of saliva.

Received for publication December 30, 1948.

<sup>1</sup> Aided by a grant from the National Foundation for Infantile Paralysis, Inc.

### METHODS

Guinea pigs were used in this study of lung edema and congestion following increased intracranial pressure. The anesthetic agent employed was sodium pentobarbital, 10 to 20 mg. intraperitoneally. A drill opening  $1\frac{1}{2}$  mm. in diameter was made in the mid-calvarium and a balloon-tipped plastic tube was carefully inserted into this opening. Wire hooks two millimeters proximal to the latex balloon were attached to rubber bands; the latter were anchored in an infero-lateral manner in order to immobilize the balloon. One and one-half cubic centimeters of air were introduced within 15 seconds through the plastic tube into the balloon. The elevated intracranial tension was maintained until the guinea pigs were guillotined 5 minutes later. A control series of anesthetized guinea pigs were guillotined, and both the experimental and control animals were held with the severed neck dependent until bleeding had ceased.

The body weight, the weight of the heart ventricles, and the total weight of the lungs minus trachea were recorded for each guinea pig. Lung weight/ventricle weight ratios and lung weight/body weight ratios were used as supplementary aids to gross examination of the lungs for a better quantitative analysis of pulmonary pathology.

### RESULTS

The data from 24 guinea pigs, which were subjected to increased intracranial pressure for 5 minutes prior to guillotining, and 26 control animals are listed in tables 1 and 2 respectively. The experimental group in table 1 displays a lung weight/ventricle weight ratio varying from 2.13 to 5.69 with an arithmetic mean of 3.61. A range from 1.74 to 3.28 may be noted in the lung weight/ventricle weight ratios in table 2 of the control guinea pigs with an arithmetic mean of 2.41. Twelve of the 24 guinea pigs subjected to elevated intracranial tension and none of the 26 control animals revealed a lung weight/ventricle weight ratio greater than 3.40. Employing the  $X^2$  4-fold table, these differences between the control and experimental animals have a probability of less than .05 per cent of occurring due to chance, indicating a statistically significant difference between these two groups with reference to lung weight/ventricle weight ratios.

Tables 1 and 2 also contain the data regarding lung weight/body weight ratios. The differences observed between the control and experimental groups are statistically significant, for the chance of probability alone giving such results is again less than .05 per cent.

Approximately one half of the guinea pigs subjected to increased intracranial pressure were found to have lung weight/ventricle weight and lung weight/body weight ratios higher than the largest value in the control group. This same 50 per cent of experimental animals displayed pulmonary edema, congestion, and hemorrhage on gross examination of the lungs at necropsy. Figure 1 presents a photograph of the lungs of a typical animal from the elevated intracranial pressure and the control groups respectively. Asterisks in the tables identify the lungs illustrated.

The effect of bilateral cervical vagotomy was subsequently studied. The guinea pigs were guillotined 5 minutes after injection of  $1\frac{1}{2}$  cc. of air into the intracranial

TABLE 1. GUINEA PIGS SUBJECTED TO INCREASED INTRACRANIAL PRESSURE

WT. OF GUINEA PIG gm.	LUNG/VENTRICLES	LUNG/BODY X 10 <sup>-4</sup>	WT. OF GUINEA PIG gm.	LUNG/VENTRICLES	LUNG/BODY X 10 <sup>-4</sup>
376	5.32	136	340	4.65	135
308	5.09	189	400	2.27	79
292	5.10	151	355	5.12	169
371	5.12	161	315	3.59	113
300	3.46	93	207	2.52	81
350	2.67	63	369	2.44	62
330	3.12	89	210	4.31	127
344	3.88	96	217	2.90	82
309	3.02	98	210	3.80	116
340	3.22	95	Mean ... 291	3.51	107
319	2.96	83			
340	2.85	81			
310	2.13	75	305	2.62	78
380	3.11	93	391	3.32	90
253*	4.03	112	280	3.68	87
Mean ... 328	3.71	108	234	2.65	83
			358	3.29	99
			210	3.16	94
			219	2.62	84
			214	2.33	75
			195	3.12	82
			Mean ... 267	2.98	86

TABLE 2. CONTROL GUINEA PIGS

WT. OF GUINEA PIG gm.	LUNGS/VENTRICLES	LUNG/BODY X 10 <sup>-4</sup>	WT. OF GUINEA PIG gm.	LUNGS/VENTRICLES	LUNG/BODY X 10 <sup>-4</sup>
303	2.42	71	297	2.37	71
272	1.86	78	341	2.47	79
320	2.08	68	338	2.26	70
249	2.39	76	370	2.56	73
251	2.89	80	336	2.42	67
312	2.35	70	340	2.31	60
294	1.74	74	329	2.34	69
261	2.53	90	284	3.28	77
237	2.31	77	376	2.16	58
314	2.74	83	340	2.70	80
286	2.27	65	382	2.32	68
246	2.29	63	279*	2.84	67
395	2.28	64	Mean 310	2.41	72
313	2.59	68			

balloon. The cervical vagi were isolated in each animal, and a silk ligature was loosely placed around each vagus nerve. A few seconds prior to the elevation of intracranial pressure, the nerves were divided in one group of 9 animals, while, in another 9 selected at random from the same lot, the vagus nerves were left intact.

Table 1 shows that 4 of the 9 non-vagotomized guinea pigs had lung weight/ventricle weight ratios greater than 3.70, whereas none of the 9 animals with bilateral cervical vagotomy presented so high a ratio. Application of the  $\chi^2$  test indicates that the probability of such findings being due to chance alone is 2.3 per cent. There is a probability of only 0.8 per cent that the differences in lung weight/body weight ratios between the sham-operated and vagotomized animals in table 1 are functions of chance. In table 1, one sees that 5 of the 9 animals with intact vagi exposed to elevated intracranial pressure show lung weight/body weight ratios higher than  $10 \times 10^{-4}$ , whereas by contrast none of the 9 in the vagotomized group shows such a high lung weight/body weight ratio. It is evident that vagotomy exerted a large protec-



Fig. 1. THE LUNGS ON THE LEFT are those of a guinea pig weighing 253 gm. after exposure to increased intracranial pressure. Those on the right are from a control guinea pig weighing 279 gm. Both animals were guillotined. The animals were representative of the groups and are identified in tables 1 and 2 by asterisks.

tive effect against lung edema. There is certainly no indication that elimination of vagal innervation of lung vessels promoted edema production.

#### SUMMARY

Pulmonary edema, congestion and hemorrhage plus abnormally high lung weight/body weight and lung weight/ventricle weight ratios were found in one-half of 24 guinea pigs subjected to increased intracranial pressure. The latter was produced by the injection of air into a rubber balloon placed extradurally through a small opening in the mid-calvarium.

Bilateral cervical vagotomy in 9 guinea pigs a few seconds prior to similar elevation of intracranial tension resulted in a significantly lesser degree of pulmonary pathology. Furthermore, a significant lowering of lung weight/ventricle weight and

lung weight/body weight ratios was observed in the 9 vagotomized guinea pigs as compared to 9 animals from the same lot with intact vagi subjected to identical cerebral trauma. In these experiments bilateral cervical vagotomy exerted a protective effect against pulmonary edema subsequent to elevation in intracranial pressure.

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# STUDIES IN THE FRACTIONATION OF LIVER: COMPOSITION OF REGENERATING LIVER AFTER PARTIAL HEPATECTOMY IN RATS<sup>1</sup>

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THE regenerating liver of the partially hepatectomized rat provides an excellent tissue for the study of the relationship between growth and chemical composition. Changes in cell structure (1, 2), lipids (3), proteins (4), nucleic acid (5, 6) and cytochrome C (6) have been determined in the whole liver tissue after partial hepatectomy. During the course of a study involving the fractionation of liver proteins (7), three main fractions having distinctive physical and chemical properties could be separated. The present investigation is concerned with the dry weight, the nitrogen and the lipid contents and concentrations of whole liver, three liver fractions and the particulate material at frequent intervals following liver lobectomy.

## METHODS

Inbred male rats of Wistar stock, 65 to 70 days old and weighing between 150 and 200 gm., were used as experimental animals. A stock diet consisting of Gaines Meal and Purina Checkers was always available. Partial hepatectomy was done according to the procedure of Higgins and Anderson (1) under pentobarbital anesthesia; approximately 70 per cent of the total liver tissue was removed. At varying intervals after operation, the animals were exsanguinated, the livers were removed, blotted free of excess blood and stored in a beaker surrounded by ice water. The fractionation of liver was begun within 30 minutes. A group of rats was killed for a given period to yield a total of about 30 to 40 gm. of liver.

The livers were ground in a small meat grinder and transferred quantitatively to a Waring Blender with three times their weight of physiological saline and stirred at slightly above 0° for 3 minutes at about 5000 r.p.m. This suspension was transferred quantitatively into a metal beaker with five times the original liver weight of physiological saline. The suspension was adjusted to pH 7.0 with 1 M sodium carbonate and stirred slowly at 0° for 15 minutes. The volume (usually between 250-350 ml.) was accurately measured. Ten ml. of this mixture were homogenized at 0° in a small Waring Blender at high speed and aliquots were removed for the

Received for publication January 17, 1949.

<sup>1</sup> The work described in this paper was done under contract between the Medical Division, Chemical Corps, United States Army, and the University of Virginia. Under the terms of this contract the Chemical Corps neither restricts nor is responsible for the opinions or conclusions of the authors.

analysis of nitrogen and total solids. The sodium chloride was taken into consideration in calculating the total solids which were determined after heating for 48 hours at  $105^{\circ}$ .

The fractionation was done on an aliquot of 90 ml. of the liver suspension. This was centrifuged in two 50-ml. Lusteroid tubes at 10,000 r.p.m. ( $12,000 \times$  gravity) for 1 hour at  $0^{\circ}$ . The residues were combined quantitatively in a single Lusteroid tube with the aid of a portion of the supernatant. The residue (A) was obtained by centrifuging at 10,000 r.p.m. The combined supernatants were dialyzed for 16 hours in a cellophane sack against running tap water maintained at about  $10^{\circ}$ , and were adjusted to  $pH\ 5.8 \pm 0.05$  with an acetate buffer (ionic strength, 0.4). The precipitate (B) which formed immediately was allowed to stand for 30 minutes at  $0^{\circ}$  and then removed by centrifuging for 15 minutes at 4500 r.p.m. at  $0^{\circ}$ . The supernatant of Fraction B was adjusted to a final ethanol concentration of 70 per cent with 95 per cent ethanol. A precipitate (C) formed immediately and after standing at  $0^{\circ}$  for one-half hour, the suspension was centrifuged at  $-5^{\circ}$  at 4500 r.p.m. for 30 minutes. All fractions were quantitatively transferred to a weighing bottle with the aid of minimal amounts of distilled water.

The remaining liver suspension was centrifuged at 1000 r.p.m. for 10 minutes and the upper two thirds of the supernatant was syphoned off. This supernatant was centrifuged at 10,000 r.p.m. for one hour and the residue (Fraction M) which contained only mitochondria and microsomes was transferred to a vial.

The 4 fractions were dried to constant weight in a desiccator over  $P_2O_5$  in *vacuo*. The fractions were kept in the cold room until most of the water was removed and the drying was continued at room temperature. The total lipid carbon and total cholesterol were determined according to methods previously described (8). Nitrogen analyses were done according to the Kjeldahl procedure, using a copper and selenium catalyst.

#### RESULTS

The 3 liver fractions may be characterized as follows: Fraction A is the saline-insoluble residue containing cells, nuclei, connective tissue, mitochondria, large microsomes and other insoluble material. Fraction B, obtained by adjusting the dialyzed saline extract to  $pH\ 5.8$ , contains appreciable amounts of nucleic acid and lipid. Fraction C is composed of the saline-soluble protein precipitated by ethanol and contains only traces of lipid and nucleic acid.

The data for the dry weights of each of the 3 fractions per liver are plotted in figure 1. The rate of regeneration for all fractions is greatest on the second, third, and fourth days after operation. The values for the sum of the 3 fractions are consistently lower than for the whole liver due to the loss of dialyzable and non-precipitable materials. The percentage distributions of Fractions A, B, and C of livers of control rats are 59, 26, and 15, respectively. Following partial hepatectomy, this ratio remains relatively constant, indicating a uniform rate of regeneration for the cellular and cytoplasmic constituents.

The nitrogen contents of the fractions of the control and regenerating livers are shown in figure 2. A pattern similar to that seen for the total solids is obtained.

The differences observed between the curves for the whole liver and the sum of the fractions may be ascribed to the nonprotein nitrogen. The percentage distributions of nitrogen of Fractions A, B, and C in the control group are 50, 31, and 19, respectively; during regeneration, this ratio remains remarkably constant despite the large increases in liver weight.

The distribution of the total lipid carbon values are shown in figure 3. During the first 3 postoperative days, the lipid carbon contents of Fraction A remain fairly uniform and increase sharply on the fourth day. Subsequently the values tend to decrease. The postoperative lipid values of Fraction B decrease slightly from the control range. Fraction C contains only traces of lipids. The average percentage distribution of lipid carbon for Fractions A, B, and C in the control group is 73, 26,

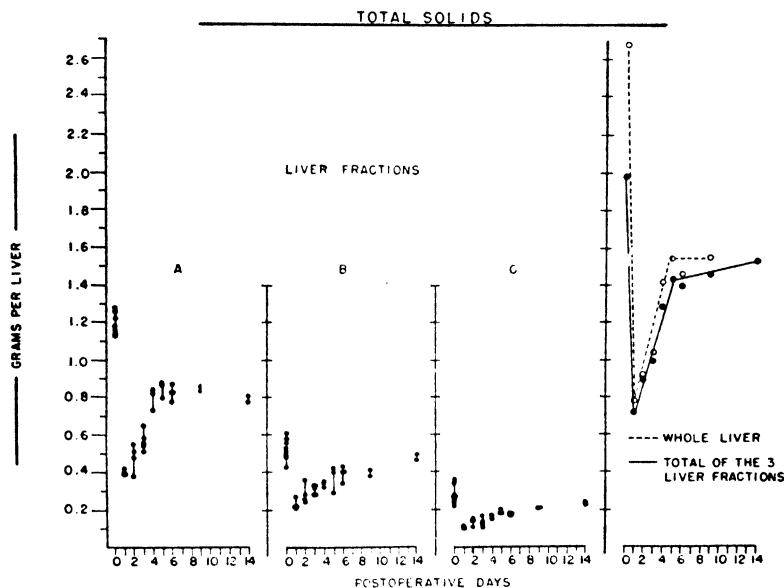


Fig. 1. TOTAL SOLID CONTENTS of 3 liver fractions after partial hepatectomy.

and 1, respectively. On the first and second postoperative days, the respective values change to 67, 31, and 2 per cent and subsequently return to the control distribution.

Cholesterol is present only in Fractions A and B (fig. 3). The cholesterol contents of Fraction A increase rapidly between the second and fifth days. The values for Fraction B decrease and remain below the control range during the experimental period. The percentage distribution of cholesterol in Fraction A and B in the control liver is 72 and 28 per cent, respectively. This ratio remains fairly constant during the first three postoperative days; during the remainder of the experiment, the ratios approximate values of 80 and 20 per cent.

Data for the percentage concentrations of total lipid carbon and cholesterol of the mitochondria are presented in figure 4. Absolute amounts are not given

since no quantitative separation of particulate matter is possible. The concentration of the total lipids is too variable for interpretation. The cholesterol concentrations show no change during the experimental period.

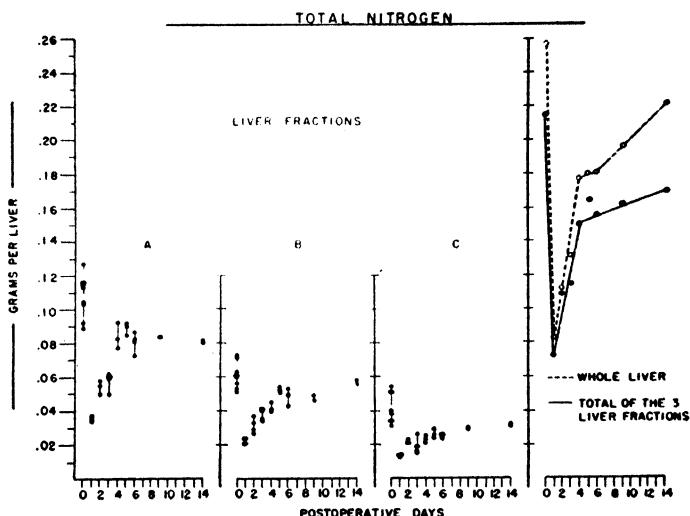


Fig. 2. NITROGEN CONTENTS OF 3 liver fractions after partial hepatectomy.

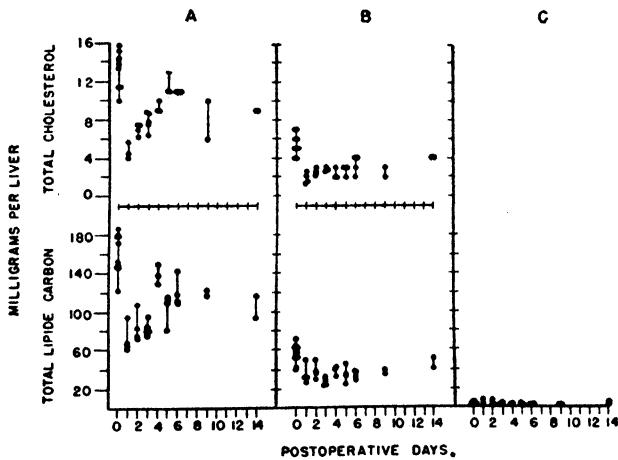


Fig. 3. LIPID CARBON AND CHOLESTEROL CONTENTS OF 3 liver fractions after partial hepatectomy.

Data are presented in table 1 which summarize the percentage changes in the amounts of total solids, nitrogen and lipid carbon at varying periods after partial hepatectomy. It is seen that the changes are approximately the same for nitrogen and total solids throughout the 14-day period of observation. The changes in lipid

## MITOCHONDRIA

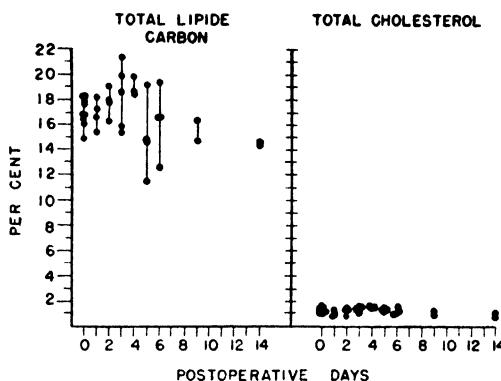


Fig. 4. PERCENTAGE CONCENTRATIONS of lipid carbon and total cholesterol in particulate components of the liver after partial hepatectomy.

TABLE 1. PERCENTAGE CHANGES FROM THE CONTROL VALUES FOR TOTAL SOLIDS, NITROGEN, AND TOTAL LIPIDS IN THE LIVER FRACTIONS AFTER PARTIAL HEPATECTOMY

POSTOPERA-TIVE DAYS	FRACTIONS							
	A			B			C	
	Total solids						NITROGEN	LIPID
0	100						100	
1	34	44	35	33	35	34	46	63
2	41	54	47	50	49	56	53	70
3	58	58	46	54	58	47	53	50
4	68	65	56	78	64	56	87	70
5	73	65	65	83	79	64	66	63
6	71	75	61	75	75	61	76	63
9	73	76	73	78	73	73	74	66
14	68	93	82	75	87	78	67	82

TABLE 2. COMPARISON OF THE LIPID CARBON CONTENTS OF FRACTION A AND B WITH CALCULATED 'CONTROLS'

POSTOPERA-TIVE DAYS	FRACTION A			FRACTION B		
	<sup>1</sup> Content	Calc. <sup>2</sup> 'control'	$\frac{1}{2} \times 100$	<sup>1</sup> Content	Calc. <sup>2</sup> 'control'	$\frac{1}{2} \times 100$
	mg.	mg.		mg.	mg.	
0	164	164		56	56	
1	73	53	138	35	20	175
2	85	81	105	39	27	144
3	84	89	95	28	32	88
4	139	123	113	39	36	108
5	105	136	77	35	44	80
6	121	123	98	35	42	83
9	119	128	93	37	41	90
14	107	123	95	46	49	94

carbon are not comparable to those observed for nitrogen and total solids during the first few postoperative days.

In order to assess the significance of the differences between the nitrogen and lipid carbon changes, the lipid carbon contents are compared with calculated 'control' values (table 2). The 'control' lipid carbon data represent values for Fractions A and B of regenerating livers in which constant lipid:nitrogen ratios of control livers are maintained. The 'control' data are obtained by multiplying the nitrogen contents of each fraction by factors representing the lipid carbon per mg. of nitrogen of Fractions A (1.52 mg.) and B (0.85 mg.) of control livers. On the first postoperative day the lipid carbon contents of Fractions A and B are 20 and 15 mg. higher than the values for the respective 'controls'. The lipid content of Fraction B remains increased (144%) on the second day while that of Fraction A returns to the 'control' level. With the exception of the fourth day, at which time there is an increase above the calculated values in both fractions, all lipid carbon contents are somewhat lower than their respective 'controls'. According to these data, the increased lipid concentration of the liver during the first two days following partial hepatectomy is associated not only with the saline insoluble components but with the saline-soluble Fraction B.

#### SUMMARY

Procedures are given for fractionating rat liver to yield: a saline-insoluble residue (A), a precipitate obtained from the dialyzed saline extract at pH 5.8 (B), and ethanol-precipitated proteins (C). Data are presented for the changes in dry weight, nitrogen, total lipid and cholesterol contents of these 3 fractions after partial hepatectomy.

During liver regeneration following partial hepatectomy, the contents of the total solids and nitrogen of each of the 3 respective fractions are closely parallel, and do not differ in their percentage distributions. Excessive amounts of lipid are present in Fraction A and B during the first and second postoperative days. The lipid carbon and cholesterol concentrations of the particulate materials remain relatively constant during liver regeneration.

The authors wish to acknowledge the able assistance of Miss Florence Jones and Miss Elizabeth A. Lenz.

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# CALORIE INTAKES IN RELATION TO THE QUANTITY AND QUALITY OF PROTEIN IN THE DIET<sup>1</sup>

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**T**HE relation between growth and food intake, and the relative merits of controlled and *ad libitum* food intakes in the conduct of nutrition experiments, have provoked much discussion. This has been especially true in studies of the nutritive value of proteins where the results could not be expressed in terms of units of the nutrient, and perhaps because gain in weight appears to be more directly related to protein deposition in the body than to the deposition of other nutrients. It is probably fair to say that the superiority of either method for the evaluation of proteins with young animals has not been proven but sufficient has been written so that it is unnecessary to reiterate the arguments here.

Hegsted and Worcester (1) found that the measurement of food intakes of rats receiving diets of constant protein content but of different nutritive value, and the subsequent calculation of protein efficiency (gain per gm. of protein eaten), did not yield significantly more information about the value of the protein than the simple measurement of gain alone. Gain and efficiency were found to be very closely correlated and appeared to measure the same characteristic of the diet. Protein efficiency was therefore discarded as a relatively useless concept. Since the diets used in that study were of constant protein content, the protein intake was proportional to the food or total calorie intake. Caloric efficiency, had it been calculated, would have shown the same correlation with gain as was found for protein efficiency. It is well known, however, that the percentage of protein in the diet has a pronounced effect upon the value obtained for the efficiency of any particular protein. With diets of varied protein content, the caloric and protein efficiency would not be parallel since the protein intake would not be proportional to the calorie intake but to the product of the food intake and the protein content of the diet. We have therefore studied the relation between calorie intake and growth when the nutritional value of the diet is varied either by changing the protein content or the quality of protein in the diet.

## EXPERIMENTAL

Six separate experiments were made at various times during a 2-year period in which 5 different proteins were fed at several levels in the diet. These included vitamin-free casein, beef protein, and 3 different samples of soy flour which had received different degrees of heat treatment. The samples and the levels incorporated in the diets are shown in table 1. The diets contained in addition to the quantity of supplement required to supply the desired level of protein, 4 per cent corn oil, 4 per cent salt mixture (2), 1 per cent Wilson's liver extract 'L', and glucose to complete to 100 per cent. Crystalline thiamine hydrochloride, 200 µg; pyridoxine HCl, 200 µg; riboflavin, 400 µg; calcium pantothenate, 1500 µg; niacin, 2500 µg; and cho-

Received for publication January 31, 1949.

<sup>1</sup> Supported in part by grants-in-aid from the Nutrition Foundation, Inc., New York City, the Milbank Memorial Fund, New York City, and the American Meat Institute, Chicago, Ill.

line chloride, 150 mg. were added to each 100 gm. of ration. One drop of haliver oil was administered weekly to each animal. Small groups of male rats weighing approximately 45 gm. housed in individual cages were fed each diet *ad libitum* for 4 weeks. Food intake was determined daily and the animals were weighed twice weekly. Four or 5 animals per group were used in the first 5 experiments while in the 6th, 24 rats were all fed the same diet containing 10 per cent of casein. Constant temperature animal rooms were not available but the temperature was usually between 24° and 26°C. Data on animals which died during the course of the experiment were discarded. In *experiment 4* almost half of the animals died for unknown reasons, probably unrelated to the diets they received.

#### RESULTS

The data have been studied in several ways. Many of these treatments appeared useless and others will be considered in a later paper. However, in considering gain in weight and calorie intakes it was soon apparent that these could not be directly related since, as discussed previously for protein (1), no account would be taken of

TABLE I. LEVELS OF THE VARIOUS PROTEIN FED

EXPER.	PROTEIN	LEVELS OF PROTEIN FED	NO. OF RATS PER LEVEL
1	Casein <sup>1</sup>	6, 8, 10, 15, 20	4
2	Beef protein <sup>2</sup>	5, 7, 9, 13, 18	4
3	Soy flour No. 1 <sup>3</sup>	5, 10, 15, 20, 25	5
4	Soy flour No. 2 <sup>3</sup>	5, 10, 15, 20, 25	5
5	Soy flour No. 5 <sup>3</sup>	5, 10, 15, 20, 25	5
6	Casein <sup>1</sup>	10	24

<sup>1</sup> Vitamin-free, General Biochemicals Inc. <sup>2</sup> Prepared from beef muscle, Wilson Laboratories. <sup>3</sup> Defatted soy flours, Archer-Daniels Midland Company, subjected to varying degrees of heat treatment. Soy flour no. 1 received least heat and no. 5 the most.

the calories required for maintenance. It appeared more logical to attempt to relate the caloric intake to the total body weight of the animal rather than to the gain in weight. The results of the analyses appear to support this supposition. Since daily food intakes are variable, mean food intakes over a considerable period of time were used and correlated with the mean body weight during the same period. Mean body weight was obtained by averaging the weekly weights.

Figure 1 is a scatter diagram of each experiment in which the logarithms of the mean calorie intake per day have been plotted against the logarithms of the mean body weight during the 4-week experimental period. While it is often not possible to show that the use of logarithms improves the correlation, probably because of the relatively short range over which the present data extend, they have been used because of the success of similar plots in the work on basal metabolism by Brody and others (3).

The appropriate regression lines were calculated for each experiment. The equations for these lines, shown in table 2, are of the type,  $y = bx + a$ , where  $y = \log$  calorie intake per day,  $x = \log$  mean weight, and  $b$  is the slope of the line. The standard error of the slope,  $s_b$ , and the standard error of the estimate,  $s_{xy}$ , are also shown. The lines for soy flour number 1 and soy flour number 2 have slopes some-

what, but not significantly, greater than the other 3 lines. None of the slopes is significantly different from any other when tested at the 5-per cent probability level. Therefore, all of the data may be combined as shown in figure 2, where they have been plotted on log-log paper. The regression line for the combined data,

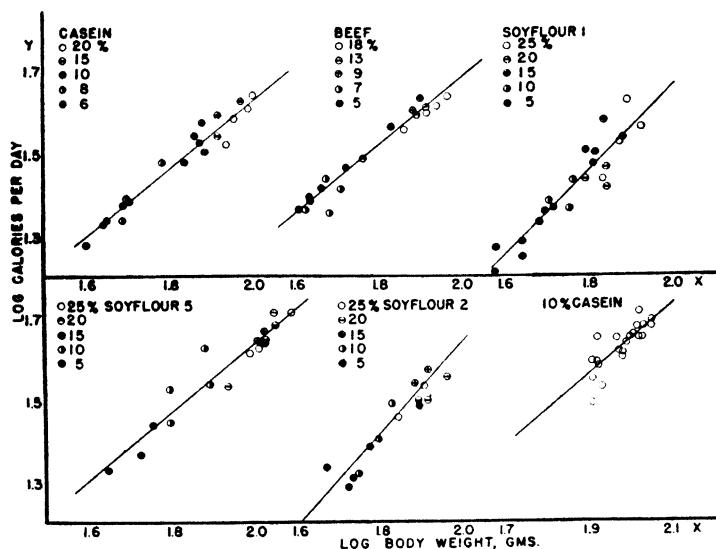


Fig. 1. SCATTER DIAGRAM showing the relation between logarithm calorie intake and logarithm body weight in the various experiments.

TABLE 2. EQUATIONS FOR THE RELATION OF LOG CALORIES PER DAY TO LOG MEAN WEIGHT FOR VARIOUS DIETS

PROTEIN FED	EQUATION OF REGRESSION LINE	r	s <sub>b</sub>	s <sub>y,x</sub>
Casein.....	$Y = \log Cal.; X = \log wt. in gm.$			
Casein.....	$Y = .843X - .0745$	.963	.017	.031
Beef.....	$Y = .823X + .0331$	.976	.043	.023
Soy flour 1.....	$Y = 1.043X - .4203$	.927	.094	.044
Soy flour 2.....	$Y = 1.059X - .4961$	.939	.107	.035
Soy flour 5.....	$Y = .847X - .0567$	.953	.069	.037
Casein (10%).....	$Y = .872X - .0956$	.792	.154	.034

r = Correlation coefficient; s<sub>b</sub> = standard error of regression coefficient; s<sub>y,x</sub> = standard error of estimate.

$\log cal/day = .882 \log wt. - .1228$ , becomes  $cal/day = .756 \text{ wt}^{.882}$  when the logarithms are removed. The relation of body weight to energy expenditure in resting rats and the basal metabolism of normal rats of various sizes is also shown for comparison. These data were obtained by Brody and coworkers (3, p. 406) by oxygen consumption measurements at 30°C. and, for animals of similar size, clearly parallel our data upon food consumption.

These data offered also a means of testing the effect of the length of time that the experiment is run upon the accuracy of the results obtained. The data from 2 of the experiments were analyzed separately for the first week, the first 2 weeks, etc.,

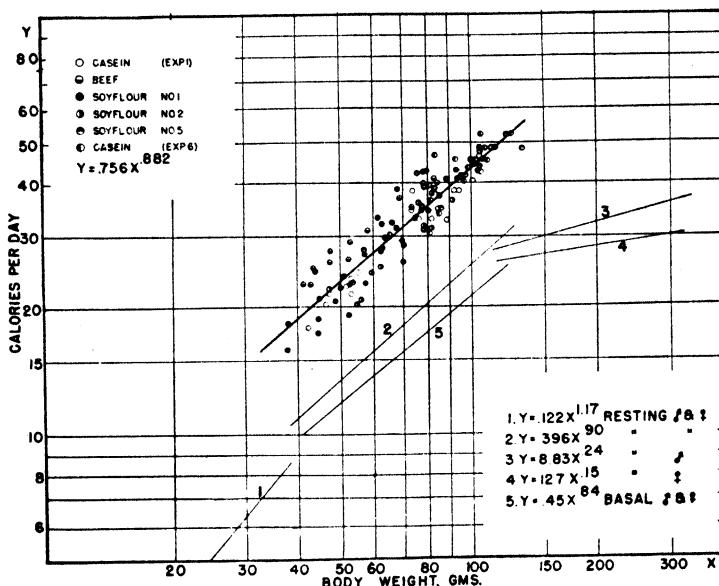


Fig. 2. COMBINED DATA FROM ALL EXPERIMENTS have been plotted to show the relation of total caloric intake to the basal and resting metabolism of rats as determined by Brody.

TABLE 3. EFFECT OF TIME ON RELATION BETWEEN LOG CALORIES PER DAY AND LOG MEAN WEIGHT AND THE ACCURACY OF MEAN GAIN

TIME PERIOD	EXPER. 1, 5 LEVELS OF CASEIN			EXPER. 6, 1 LEVEL OF CASEIN			C		
	wt. vs. Calories			wt. vs. Calories					
	wks.	r	b ± sb	sb/b, %	r	b ± sb	sb/b, %		
0-1					.651	.806 ± .2154	27.6	15.1 ± 5.68	37.5
0-2	.935	.891 ± .0793	8.85	7.54	.824 ± .1646	20.0	37.5 ± 8.98	23.9	
0-3	.957	.811 ± .0183	2.21	.802	.852 ± .1456	18.1	54.8 ± 10.10	18.6	
0-4	.963	.842 ± .0176	2.09	.792	.872 ± .1539	19.4	84.8 ± 16.40	19.3	
2-4	.863	.876 ± .1280	14.60						

C = Coefficient of variation of gain; r = correlation coefficient; b ± sb = regression coefficient ± standard error.

as shown in table 3. The standard error of the slope of the regression line obtained in each case has been calculated as per cent of the slope. Also, in experiment 6, where data upon a larger group of animals receiving the same diet were available, the mean gain during the various time periods, the standard deviation, and the coefficient of variation were calculated. Each criterion indicates that maximum

accuracy has been obtained after 3 weeks although the improvement over the 2-week data is only slight.

#### DISCUSSION

The data taken together as in figure 2 or for the individual experiments as in figure 1 indicate that, within the limits of error of the data, the mean food intake per day during the period of study was proportional to the basal metabolism or weight to approximately the 0.9 power. Since the same relation was found during the first 2, 3, or 4 weeks of the period, it would appear that the mean daily food intake at any time during the first 4 weeks was also proportional to this power of body weight. It is realized that as a matter of fact, daily food intakes are highly variable but it is convenient for the purposes of discussion and analysis to speak of daily food intakes. The constancy in the relation of food intake to basal metabolism appears to hold regardless of the rate of gain or the reason for the differences in the rate of gain, since in most of the experiments the rate of gain was controlled by the amount or kind of protein in the diet but in one experiment (*exper. 6*) the differences in gain were due to differences inherent in the animals themselves, all animals receiving the same diet.

It may be argued that the regression lines showing the relation of calorie intake to body weight in figure 1 have not been proven to be the same and this must be admitted. It can be said that the data, as they stand, do not indicate significant differences in the relation in the 6 experiments and even if minor differences due to diet be admitted, the more important fact remains that the food intakes are approximately parallel to the basal metabolism. The total calorie intake is of course considerably above the calories expended by resting rats or rats in the basal state. The difference between our calculated line and that of Brody (fig. 2) may be accounted for as difference in temperature at which the studies were done, activity in our animals, energy consumed but undigested in our animals, etc. From the data of Swift and Forbes (4) it would appear that the basal metabolisms of our animals would be approximately 20 per cent above Brody's data because of the differences in temperature. A comparison of the two lines seems valid since differences should be relative throughout the study although due to several factors just mentioned.

Assuming that the mean caloric intake is a constant percentage above the mean basal metabolism for all of the animals regardless of the rate of gain, a rough estimate of the distribution of the calories eaten may be made as shown in table 4. The computation is subject to criticism since possible differences in body composition have not been considered. However, the data of Bosshardt *et al.* (5) indicate that differences in body composition may be expected to be slight, contrary to the conclusion of Mitchell and Carmen (6). The difference in caloric intake and basal metabolism gives the calories available for growth, activity, etc. These are essentially constant when expressed as percentage of the basal calories (column 5). The gain times 2.5 cal/gm. gives the calories deposited as tissue, assuming as indicated above that the composition of the gains is not markedly different. The efficiency of the calories thus available for gain is shown in column 9. As concluded in a previous publication (1) the efficiency is very closely related to the rate of gain. Finally

the calories used neither as basal metabolism or gain must be burned or metabolized, presumably in activity (column 10). These appear to be relatively constant in terms of calories but decrease rapidly when compared to the total calories available (column 11). Thus, in the relative sense, animals which are unable to grow are wasteful of calories even when the calories required for basal metabolism are subtracted. We believe them to be more active and irritable although no measurements are available. Mitchell and Carmen (6) concluded that the calorie increment due to activity in normal rats is relatively small and this was true in the well nourished rats.

The total calorie distribution may be represented by the following equation: total calorie intake =  $aWt^{.8 \pm .9} + 2.5$  gain in gm. + 7 calories. Such an equation, however, is of little value as an aid in considering the factors which govern food intake. It implies that the animals eat enough calories to meet basal needs, plus enough to meet the gain which the particular diet is capable of supporting, plus 7

TABLE 4. CALCULATED DATA FROM FIG. 2 SHOWING THE CALORIE INTAKE AND THE DISPOSITION OF CALORIES IN ANIMALS GAINING AT VARIOUS RATES

1 MEAN WT. gm.	2 BASAL METABOL- ISM Cal/day	3 TOTAL INTAKE Cal/day	4 CALORIE INTAKE ABOVE BM 3 - 2 $\frac{3-2}{2} \times 100$	5 % of BM	6	7	8	9 EFFICIENCY $\frac{8}{4} \times 100$	10 HEAT PROD. ABOVE BM 4-8 Cal. % of BM
					Total	Daily	Daily		
45	13.78	21.61	7.83	56.8	0	0	0	0	7.83 56.8
50	15.03	23.73	8.70	57.8	10	.35	.89	10.2	7.81 52.0
65	18.75	29.89	11.14	59.5	40	1.43	3.58	32.1	7.56 40.3
75	21.13	33.90	12.77	60.5	60	2.14	5.35	41.9	7.42 35.1
95	25.80	41.76	15.96	61.8	100	3.57	8.93	55.9	7.03 27.2

calories. We do not conceive of an animal eating in this fashion and propose as an alternative, and to us more logical, interpretation that the food intake of an animal is governed by means yet unknown at a certain percentage above its normal basal metabolism. This amount of food once eaten must be disposed of. Gain will result to the extent that the protein, vitamin and mineral needs are met by this amount of food. The remainder must be used, presumably as activity. If the food eaten does allow growth, the body size will increase, the basal metabolism will be increased, and the food intake on succeeding days thus increase.

Other explanations are perhaps as valid, but these suggestions may lead to some advance in the provoking question: "Why does an animal eat the amount of food that it does under various dietary conditions?" Those who have followed the arguments of *ad libitum* versus paired feeding during the years are aware that this is more than the familiar 'chicken and egg' proposition, and is often of fundamental importance in the interpretation of feeding experiments.

Whether few or many feeding studies can be interpreted upon a similar basis remains to be determined. Changes in basal metabolism as a result of the deficiency

would obviously negate the theory but such changes were not observed in a similar study with rats fed various levels of protein by Forbes *et al.* (7). Also deficiencies which directly affect appetite, such as thiamine deficiency, could not be expected to follow the trends exhibited by the protein deficient animals in this study. As Kleiber (8) has pointed out, the efficiency of energy utilization during dietary deficiency may be lowered by decreasing the food intake, increasing the basal metabolism, or increasing the calorigenic action of the food. A decision as to whether the first or last of these 3 has occurred in any particular study will require careful consideration of the baseline to which the food intake is compared. Also, to state that the calorigenic action of the food is increased implies a positive action of the food. In the present study, it is true that a larger proportion of the calories eaten has been expended as heat, but we do not interpret this as a direct action of the food or the deficiency but merely as a necessary consequence once food which does not allow growth is eaten.

The question of the baseline to which metabolic measurements and food intakes, etc. should be compared is of considerable importance. The writings of Brody (3) and Kleiber (9, 10) contain repeated warnings against the use of surface area as the baseline and these authors have thoroughly discussed the reasons for this conclusion. Both of these authors find that in normal adult animals of various species the basal metabolism varies approximately as weight to the 0.7 to .75 power. Kleiber (10) appears to be inclined to utilize the same unit in various experiments utilizing animals of various species and ages, but the data for various species presented in his paper lend considerable support to the belief that this unit may not be applicable within any one species. The extensive data compiled by Brody (3, chap. 14) indicate marked changes in the relation of metabolism to body weight with age. In rats weighing from 40 to 100 gm. the basal metabolism apparently varies approximately with weight to the 0.84 to 0.9 power, as already indicated, while in adult male rats it was found to vary with weight to the 0.35 power. Evidence being accumulated in our laboratory (McPhee and Hegsted, unpublished) suggests that food intakes in adult animals likewise vary with approximately this power of body weight,  $Wt^{.35}$ . The recommendation of Brody and Kleiber that surface area be discarded as a unit in metabolic studies, and where ever possible the true power of body weight which corresponds to metabolism be used, should receive the recognition it deserves.

In this regard it should be noted that according to Brody, the basal metabolism in relation to body weight changes at or shortly after puberty. Studies continued for long periods may result in some of the animals reaching weights where they are no longer comparable to other animals started at the same time. Since in the present studies apparently maximum accuracy was obtained within 3 weeks, long feeding experiments would appear to offer little advantage for many problems and may actually distort the results.

Finally Kleiber (10) concluded, "two animals may be regarded as being upon the same level of food intake when the rate of intake of metabolizable energy is the same multiple or the same fraction of the standard metabolic rate." Our *ad libitum* fed animals would appear to satisfy this condition even though they were growing at

markedly different rates. If this be true, then the main argument for paired-feeding, that the food intake of animals receiving *ad libitum* feeding is not controlled, would appear to be based on false assumptions. It is clear that the food intake is controlled and, whatever the mechanism may be, these data suggest that it is controlled in proportion to metabolic rate.

#### SUMMARY

Twenty-five different diets in which the quantity and quality of protein were varied were fed to young male rats for a 4-week period. Analysis of the data upon food intake and body weights suggests that the mean daily calorie intake varied as the mean body weight raised to the 0.88 power. Animals receiving the same diet but which varied in rate of gain and in calorie intake because of inherent differences in the rats themselves showed a similar relation between food intake and body weight.

Published results by Brody indicate that the basal or resting metabolism of rats of this size also varies with body weight raised a power near this value (0.84-0.9). It therefore appears that the mean calorie intake was a constant percentage above the mean basal metabolic rate during the experimental period, regardless of the rate of gain or the cause of this difference in gain, i.e., inherent differences or differences in the nutritive value of the diet. It is suggested that the food intake of the animals is controlled by some means at a relatively constant per cent above the basal metabolism. If the food thus consumed contains adequate nutrients for growth, the animal grows, increases its basal metabolism, and thus its food intake upon succeeding days. If the food eaten does not allow growth, or only limited growth, the remaining calories must be consumed, probably in activity. The efficiency of the animal unable to grow is thus markedly diminished even when the calculation is made only upon the calories above those required for basal metabolism.

We are indebted to Corn Industries Research Foundation, New York City; Merck and Company, Inc., Rahway, N. J.; Sheffield Farms Company, Inc., New York City; Wilson Laboratories, Chicago, Ill.; and Gaines Division of General Foods Corporation, Hoboken, N. J. for generously supplying us with materials.

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# EFFECTS OF ATROPINE ON FOOD INGESTION AND WATER DRINKING IN DOGS

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**A**N ASSOCIATION of belladonna with anorexia has been reported by Rivera Pérez (1). Montgomery (2) reported that large doses of atropine did not affect significantly the water intake of dogs with or without salivary glands. Also it was observed that there was no change in the two-hourly intake of water after injection of the drug. No change in water intake was observed with pilocarpine. In another study Montgomery (3) observed that total extirpation of the salivary glands in dogs did not result in a decreased water intake. It was concluded that it was improbable that the salivary glands were a major factor in thirst regulation. Steggerda (4) in a study of the relation of thirst to dryness of the mouth in a human subject concluded that water intake is not necessarily related to a dry mouth. Studies of salivary conditioning in atropinized dogs and of pilocarpine conditioning were made by Finch (5, 6). Also the effects of atropine, pilocarpine and other drugs on oral and pharyngeal mucous secretion were reported by Montgomery (7).

The following experiment is a study of the effects of several doses of atropine sulfate on food ingestion and water drinking in dogs.

## METHODS

The experiment was divided into four periods. Six adult female dogs served as subjects. The animals were confined separately to cages. A block chow (Purina) was administered as the only source of solid food in all periods. The dogs were accustomed to this diet. Drinking water was available in the cages.

Four dogs were used in Period I. Each animal was injected intramuscularly every other day with .3 mg. atropine sulfate and on alternate days with isotonic sodium chloride solution. This routine was interrupted one day in every 7 days when the animals were not injected but had food and water available for the usual length of time. The interval of feeding was 45 minutes and began 30 minutes after the injection. The animals were fed at approximately the same time each day. The volume of water drunk during the interval of feeding was recorded. Likewise the volume of water drunk over the remainder of the 24-hour interval from a liter of water placed in the cage was recorded. The period consisted of 20 days on 10 of which the animals were injected with atropine. The data of this and subsequent periods are expressed in daily averages in table 1. The experiment was conducted in Period II identically as in Period I, except that the animal received 1 mg. of pilocarpine hydrochloride in place of atropine sulfate. The pilocarpine was used as

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Received for publication December 31, 1948.

an additional control on the atropine, and at the same time similar measurements were made on the effects of this drug in the particular dose used. A few averages in Period II are based on 8 and 9 days. Room temperature reading was recorded daily.

The length of Period III was 40 days. This period consisted of 8 intervals of 5 days each. Each interval was separated by 2 days. In the first 4 intervals, consisting of 20 days, each animal was injected with isotonic sodium chloride solution

TABLE I. AVERAGE VALUES OF DRY FOOD INGESTED AND WATER DRUNK

DOG	DRY FOOD INGESTED		WATER DRUNK IN FEEDING INTERVAL		WATER DRUNK DAILY	
	A	B	A	B	A	B
<b>PERIOD I: A, sodium chloride control; B, .3 mg. atropine sulfate</b>						
I	144	72	47	4	509	294
2	171	96	78	10	509	372
3	182	98	109	5	731	479
4 <sup>1</sup>	194	213	104	103	707	792
<b>PERIOD II: A, sodium chloride control; B, 1 mg. pilocarpine hydrochloride</b>						
I	176	153	51	53	623	498
2	182	209	91	94	642	751
3	192	209	90	101	837	847
4 <sup>1</sup>	218	210	119	88	852	916
<b>PERIOD III: A, sodium chloride control; B, .6 mg. atropine sulfate</b>						
5	128	58	63	3	458	278
6	466	267	208	25	2005	1137
2	149	122	41	13	477	497
3	228	133	127	5	956	586
<b>PERIOD IV: A, sodium chloride control; B, 1.5 mgs. atropine sulfate</b>						
I	190	4	117	15	659	56
2	202	32	95	11	678	161
3	259	20	123	11	1016	144
4 <sup>1</sup>	212	255	195	180	875	1047

<sup>1</sup> Control.

one hour before feeding time which lasted one hour at approximately the same time each day. Drinking water was available during the interval of feeding and during the remaining 23 hours. The last 4 intervals of 5 days each were conducted similarly excepting each animal was injected intramuscularly with .6 mg. atropine sulfate instead of the placebo solution of saline. The same measurements were made in Period III as in Periods I and II.

In the last phase of the experiment, Period IV, 3 of 4 dogs were injected each daily with 1.5 mg. atropine sulfate for an interval of 6 days. Then the same animals

were injected with isotonic saline for another 6 days. Otherwise the routine of this period was identical with that in Periods I and II. The fourth animal served as a control. Body weights were recorded at the beginning and end of each of these intervals.

#### RESULTS AND DISCUSSION

*Dry Food Ingestion.* The injection of the .3 mg. atropine sulfate was accompanied by a pronounced food intake inhibition in the animals. The dry-food ingestion of dogs 1, 2, and 3 in the atropine interval in Period I was 50, 56, and 54 per cent, respectively, of that in the control interval. The ingestion of the control animal at the same time was 110 per cent. The average dry-food ingestion of the identical animals in the interval when 1 mg. pilocarpine hydrochloride was injected was 87, 115, 109 and 96 per cent, respectively, of that in the control interval in Period II. It is concluded from these data that the atropine sulfate exerted a real food ingestion inhibitory effect. The pilocarpine hydrochloride seemed to have no significant effect on the quantity of food eaten. The average daily room temperature in the control interval in Period I was 21°C., while that in the atropine interval was 19°C. The average room temperature in each of the intervals in Period II was 20°C.

The dry-food ingestion of dogs 5, 6, 2, and 3 in Period III when .6 mg. atropine sulfate was injected was 45, 57, 82 and 58 per cent, respectively, of that in the control interval. The decrease in dog 2 was not as marked as in Period I. Again here it seems there was a definite inhibitory effect of the atropine on food ingestion. The average daily room temperature in the control interval in Period III was 21°C., while that in the atropine interval was 17°C. The lowered room temperature in the atropine interval resulted probably in the ingestion of more food by the animal than if the temperature were higher.

The most marked inhibition of food intake occurred in Period IV when the dogs were injected with 1.5 mg. atropine sulfate. Dogs 1, 2, and 3 ingested only about 2, 15 and 8 per cent, respectively, of the dry food ingested in the control interval. The control animal ingested about 120 per cent. The average room temperature in the sodium-chloride interval was 23°C., while that in the atropine interval was 19°C. The marked decrease in dry-food ingestion after the drug was administered occurred in spite of the drop in average room temperature.

It was observed in Period IV that the atropine was still very effective on the sixth day of administration. However, release after cessation of the drug was very prompt. The animals regained their controlled eating patterns practically at once. In the 6 days of atropine administration in Period IV dogs 1, 2, and 3 lost about .9, .6, and .7 kg., respectively. The control animal lost about .3 kg. in the same time. The former three animals regained about .5, .3, and .5 kg., respectively, in the control interval. The control animal's weight increased about .1 kg.

The food intake inhibiting effect of atropine is evident in these experiments. The question rises as to how the atropine produces this inhibition. Perhaps the mechanism of inhibition may operate at some central nervous system level. Such a level has been suggested for the mechanism of d-Amphetamine-induced decrease in appetite (8, 9).

*Water Drunk.* The effects of the atropine on the volume of water drunk in the feeding interval and that drunk daily in Period I can be deduced from the data in table 1. There was a marked inhibition of drinking in the feeding interval after the drug had been injected. These data seem to be somewhat in disagreement with those of Montgomery (2) who reported that there was no change in the 2-hourly intake of water after injection of atropine. Conditions were not exactly the same in both studies, however. In the present experiments the measurement of water intake after injection was made during the only time when food was available, as described earlier.

The water drunk daily by the atropinized animal was much less than that of the same animal in the control interval, as shown in table 1. Yet roughly the ratio of water drunk daily to dry food ingested in the control interval was disturbed but little by the administration of the atropine or pilocarpine. On this basis, it is assumed that total daily drinking was changed little by either drug. However there was some indication that the atropine may have stimulated the drinking of water slightly over the 24 hours. The same observations were made in Period III and IV with respect to the effects of the atropine sulfate on water drunk in the interval of feeding and daily.

The question rises as to what extent the urge to drink may be modified by salivation. It may seem that the probable decrease in salivation in the animal as a result of injection of the atropine might have resulted in increased drinking of water. However, there was no evidence of this at least several hours after administration of the atropine. In fact, there was an inhibition temporarily. These results and the work of others (2-4) indicate that the salivary glands may not be the primary regulator of water intake. Perhaps the mechanism of thirst operates at some central nervous system level.

#### SUMMARY

Dogs were injected with atropine sulfate and the effects of the drug in the doses used were observed on ingestion of food and drinking of water. The atropine exerted a real food ingestion inhibitory effect. Also there was generally an inhibition of water drinking immediately after injection of the atropine as explained, although there may have been a slight stimulation of water drinking over the 24 hours. The mechanism of the inhibition of food intake and temporary drinking of water by the atropine is not apparent. The pilocarpine in the quantity used produced no significant changes.

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# ESTIMATION OF RELATIVE VELOCITIES OF PLASMA AND RED CELLS IN THE CIRCULATION OF MAN<sup>1</sup>

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**F**ÄHREUS (1) on the basis of variations observed in the concentration of cells and plasma in blood flowing through glass tubes of varying diameter, concluded that the velocity of the cells must exceed that of the plasma. This hypothesis received confirmation from *in vivo* experiments conducted by Dow, Hahn and Hamilton (2), who injected red cells tagged with radioactive iron, suspended in plasma stained with Evans blue dye (T-1824), into the superior vena cava of a dog; determination of the relative radioactivity of, and plasma dye concentrations in, aliquot specimens of blood drained from the left heart indicated that the erythrocytes moved slightly faster than the plasma.

The present communication describes a method for the separate estimation of cell and plasma velocity through an isolated segment of the peripheral circulation in man. Included are the results of 6 experiments, demonstrating that erythrocytes move at a more rapid rate than plasma.

## METHODS AND RESULTS

By injecting instantaneously a mixture of traceable red cells and plasma at one point in the circulation and collecting samples at frequent intervals from another the relative velocities of the cells and plasma were determined in their transit past the point of sampling. In order to recover the donor cells and plasma in concentrations sufficiently high to permit accurate measurement of small samples a single capillary bed, that of the forearm, was selected for study. The injections were made into the brachial artery and the collections were taken from an antecubital vein, the circulation to the hand being excluded meanwhile by a pneumatic cuff about the wrist inflated to pressures above systolic.

The donor cells were selected for their failure to agglutinate when tested with anti A, B or M grouping serum, the recipient cells, on the other hand, being practically completely agglutinated by the test serum. Recipients were selected as test subjects only if less than 0.4 per cent of their red cells failed to agglutinate after exposure to the test serum employed. By contrast a sufficient quantity of donor cells was injected to reach peak concentrations of 20 per cent in the collected blood samples. A description of the technique and a statistical analysis of its accuracy has been reported by Young and his co-workers (3). The error of the method was judged to be  $\pm 2.2$  per cent (S. D.  $\pm 2$ ) (4).

Received for publication January 19, 1949.

<sup>1</sup> This investigation was supported in part by the Squibb Institute for Medical Research, New Brunswick, N. J.

In studying the velocity of the plasma, advantage was taken of the fact that the dye T-1824 is bound firmly and instantaneously to the plasma proteins. Three parts of a concentrated suspension of red cells in plasma were mixed in a sterile stoppered flask with one part of a 0.1 per cent solution of T-1824 in normal saline. Usually 3 cc. of this mixture was taken up in a 5-cc. syringe and injected into the brachial artery.

With the patient supine the brachial artery was punctured with an 18-gauge needle through novocainized skin. The patency of the needle was maintained either by connecting it through a Hamilton manometer to a reservoir containing 5 per cent sodium citrate or by means of a stylet and repeated flushing with heparin solution. The venous needle was 13- or 15-gauge and was directed against the stream in a large vein primarily draining deeper tissues. Its patency was maintained by the slow infusion of isotonic saline through a three-way stopcock. A short length of nylon catheter was connected to the other end of the three-way stopcock to facilitate the collection of samples in Kahn precipitin tubes. Two drops of a mixture of potassium and ammonium oxalate dissolved in saline (5) were placed in each test tube to prevent coagulation and minimize hemolysis.

After occluding the circulation to the hand the valve of the three-way stopcock was turned so that blood was allowed to run from the vein through the catheter connector into a 10-cc. test tube until approximately 5 cc. of blood had been collected. The end of the catheter connector was then moved to the first of the series of precipitin tubes and the 'blank' or reference sample was obtained. While the latter sample was being collected an assistant connected the syringe containing the mixture of donor cells and dyed plasma to the arterial needle and injected the contents of the syringe.

The injection, which was almost instantaneous, was accompanied by a verbal signal to a second assistant who began to call off second intervals from a stopwatch. The end of the catheter connector was then moved from tube to tube at 2-second intervals until the timed collection period was completed immediately following which the tubes were corked and the blood mixed with the oxalate by means of gentle inversion of the tubes. When the collection period was longer than 30 seconds more than one test tube rack was used in order to prevent clotting since the tubes in the first rack could be corked and inverted, while the samples in the second rack were being collected. After flushing the three-way stopcock and its connections a duplicate series of determinations could be carried out.

If venous blood flow was too sluggish to permit the collection of sufficiently large samples or if hemolysis occurred the samples were discarded. It was essential to place both needles well within the lumen of the respective blood vessels and to prevent the venous needle from becoming obstructed through contact with the vein wall. In several instances adequate blood flow was obtained by creating reactive hyperemia in the forearm by means of a blood pressure cuff placed high on the upper arm. After the circulation to the upper extremity had been occluded for 5 minutes the pressure in the arm cuff was released and the test was carried out during the hyperemic period.

The cell counts were performed by the selective agglutination techniques of

Ashby (6) or Landsteiner and his co-workers (7), depending on the blood type of the donor, using desiccated rabbit serum to agglutinate the recipient cells. The samples were then centrifugalized at a speed of 3000 R.P.M. for one hour, and the plasma pipetted off. The plasma samples which were heavily dyed with T-1824 were diluted by pipetting 0.2 cc. of each plasma sample into 1.0 cc. of normal saline. Using as a reference the diluted plasma sample collected immediately prior to the injection of the cell-dye mixture the density of the remaining samples were determined at a wavelength of 620 millimicrons in the Coleman Junior spectrophotometer.

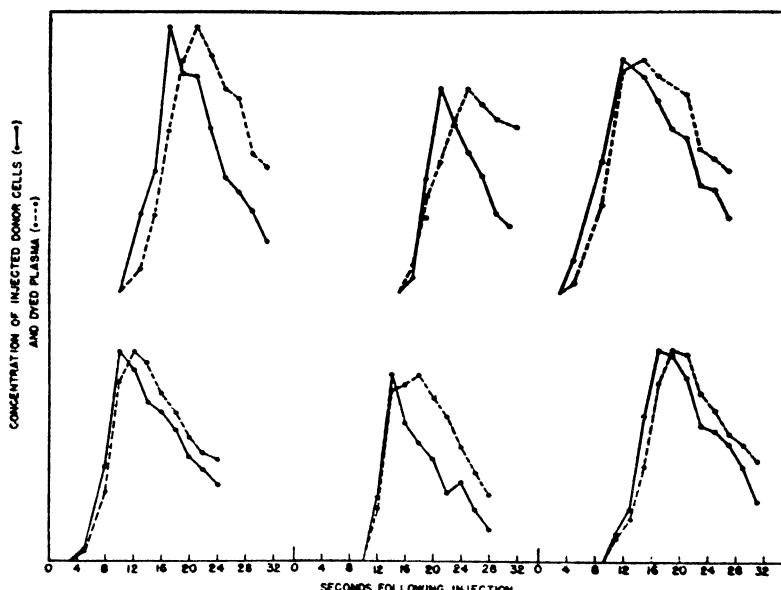


Fig. 1. TIME CONCENTRATION CURVES of donor cells and dyed plasma in 6 separate experiments on 4 subjects. The concentration scales have been adjusted in each case so that the peaks of the dye and cell concentrations fall at equal points on the ordinate.

Analysis of the curves of concentration of the injected cells and dyed plasma in six separate determinations in 4 subjects disclosed that in every case the mean velocity of the cell mass was greater than that of the plasma mass (fig. 1). Although the appearance time of donor cells and dyed plasma was simultaneous in most instances the concentration of cells had reached its peak and was already declining when the concentration of dyed plasma was still rising. In addition, the gradient of both the rising and the falling phases of the cell concentration curves usually was steeper than that of the dye curves further indicating a more rapid transit of the cell mass.

#### DISCUSSION

The observations that the mean velocity of the cell mass is greater than the mean velocity of the plasma mass are consistent with the laws of Poiseuille (8) concerning

the movement of fluid in tubes and of Fåhreus (1) concerning the movement of cells in the blood. The velocity of flow in the blood vessels is laminar in character because of the frictional resistance of the vessel walls and intramolecular friction, being greatest in the center of the stream and least at the periphery. This streaming effect is amplified by two factors: 1) the viscosity of the blood and 2) the relatively small diameter of the individual capillaries. Laminar flow would be most pronounced in the capillaries where a given volume of plasma is exposed to a greater frictional surface area than in the large vessels. The velocity gradient between this slow moving plasma at the capillary walls and the fast moving plasma in the center of the stream, therefore, would be large.

Further, since by Bernoulli's law (9) the lateral pressure of the fluid in a tube is inversely proportional to the velocity of flow, the larger the difference in velocity between the center and the periphery of the stream, the greater will be the gradient of lateral pressure. Hence, in the minute vessels such as the capillaries where the velocity gradient is very large, the pressure gradient from the axis to the periphery of the stream also will be large. Thus, in the capillaries the velocity will be greatest in the center of the stream but the pressure will be lowest in this region. As a consequence the cells which are in effect particles floating in a liquid menstruum will be forced into the area of least pressure, the central stream, which is also the region of greatest velocity. Thus, the greater mean velocity of the cell mass may be explained readily in terms of established laws of hydraulics.

That the appearance time was the same for the plasma and the cells may be explained by the fact that the small segment of the plasma mass occupying the central stream will traverse the capillary bed at the same speed as the fastest moving cells.

Since the characteristics of laminar flow are most pronounced in the vessels of smallest diameter it is evident that in the capillaries a considerable proportion of plasma will lose velocity through frictional resistance with the capillary walls whereas the red cells will move ahead in the central stream. Hence, as pointed out by Fåhreus (1) the proportion of cells to plasma will be less in the capillaries than in the large vessels. These data, therefore, support the conclusions of Stead and Ebert (10) and of Gibson and his co-workers (11) that the hematocrit of the capillary blood is significantly lower than the hematocrit of blood flowing in larger vessels. The falsely high hematocrit in the large vessels may explain at least in part the fact that the total blood volume calculated from the plasma volume and such a hematocrit usually is significantly higher than the total blood volume calculated from the red cell volume and plasmaticcrit (11).

#### SUMMARY

A method is described for determining the relative velocity of identified plasma and cells during a single circulation through an isolated peripheral segment of the circulation (the forearm) in man. In 6 experiments the mean velocity of the cell mass was found to be perceptibly greater than the mean velocity of the plasma mass, thus demonstrating in the circulation of man the principles governing the velocity of particles in a stream subjected to laminar flow.

The authors thank Dr. Robert W. Wilkins for helpful advice and criticisms.

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# TETRAETHYLMAMMONIUM AS AN AID IN THE STUDY OF CARDIOVASCULAR REFLEXES<sup>1,2</sup>

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**S**INCE tetraethylammonium (TEA) was shown to block transmission in autonomic ganglia (1, 2), it has been widely used as a means of assessing vasomotor tone in the human subject. Simultaneous measurements of arterial pressure and of blood flow in the extremities or in the kidneys have made possible an estimate of vasomotor tone in these vascular areas in normal and diseased subjects (3, 4); attempts have also been made to estimate the degree of vasomotor tone in hypertension in terms of the depressor response to the drug (5, 6).

Although the term 'vasomotor tone' may be interpreted to mean neurogenic constrictor tone to both arteries and veins, it is often used to signify only arteriolar vasoconstrictor tone, and therefore the neurogenic component of peripheral resistance. Except for regulation of blood flow to various organs for specific purposes (as, for example, to the skin for regulation of heat dissipation), cardiovascular reflexes are primarily adapted for the maintenance of arterial pressure. It is probable that when the so-called vasomotor center is called upon to prevent a fall of arterial pressure, all available pressor mechanisms are utilized. These mechanisms, in addition to arteriolar constriction, are cardioacceleration, probably also inotropic cardiac stimulation, and venous constriction; the three last devices, of course, can affect arterial pressure by increasing cardiac output. The integrated action of all accomplishes the desired object: maintenance of arterial pressure.

TEA should be capable of interrupting *all* autonomic influences on the cardiovascular system: cardioaccelerator and cardiodecelerator, vasoconstrictor and vasodilator, and venomotor. Although the response to this agent would, at first thought, appear to be too complex, it has nevertheless proved useful as a tool in the study of circulatory reflexes.

In the experiments described below, TEA has been used to measure reflex alterations of vasomotor tone induced by various procedures in anesthetized dogs.

## METHODS

Dogs of both sexes, ranging in weight from 6 to 24.5 kg., were prepared under chloralose or thiopental-barbital anesthesia. Arterial pressure was recorded with

Received for publication December 17, 1948.

<sup>1</sup> This investigation was supported by a grant from the Life Insurance Medical Research Fund.

<sup>2</sup> Presented in part before the Central Society for Clinical Research, Chicago, 1946, and the XVII International Physiological Congress, Oxford, 1947.

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a mercury manometer. Femoral and renal arterial flow were recorded in certain experiments by means of a differential manometer (7). In the femoral artery the pressure decrement across a stainless steel cannula both ends of which were inserted into the artery was measured by means of a rubber membrane manometer, each side of the manometer capsule being attached to one of the side-tubes of the cannula. For convenience in the registration of renal blood flow, the left renal artery was connected to the left common carotid artery by siliconed cannulas and plastic tubing; the resistance unit of the differential manometer was inserted in the perfusion line. Coagulation was prevented by continuous infusion of heparin. Cardiac output was measured in open-chest dogs by means of a Henderson cardiometer and a spirometer-type volume recorder. In these experiments right auricular pressure was recorded with a water manometer.

Changes in vasomotor activity were induced by carotid occlusion, vagal section, carotid denervation, carotid sinus nerve stimulation, injection of pressor and depressor drugs, hemorrhage, plethora, and asphyxia. Tetraethylammonium chloride was injected either in single doses during the sustained application of one of the above stimuli, or given by continuous infusion followed by the brief application of the various procedures. When TEA was given by injection, doses were used which produced maximal depressor responses during control periods; when given by continuous infusion, it was given at a rate double that shown to prevent completely the pressor response to carotid occlusion. In most experiments a primary dose of 5 mg/kg. followed by infusion of 12 mg/kg/hr. prevented carotid pressor reflexes; an infusion rate of 20 to 30 mg/kg/hr. was then used for subsequent procedures. Asphyxia was produced in various experiments by respiration of a mixture of 90 per cent N<sub>2</sub>, 5 per cent O<sub>2</sub>, and 5 per cent CO<sub>2</sub>.

#### RESULTS

*Cardiac Output.* Although TEA produces vasodilatation in the femoral bed of the anesthetized dog (1), it also exerts a positive inotropic action on the heart in sufficiently high concentration, and it was not known to what extent cardiac output changes might contribute to or modify the action of the drug on arterial pressure. To determine the relative importance of this factor, cardiac output was measured by the oncometer method in 9 experiments (table 1).

In dogs under barbiturate anesthesia, TEA produced a decrease of arterial pressure and heart rate. Maximum responses were obtained with doses of 2 to 4 mg/kg. Cardiac output was never significantly diminished in spite of the decreased heart rate. In a few cases moderate increases occurred, but in general the change of arterial pressure reflected adequately the change of peripheral resistance. Venous pressure was not altered significantly.

In animals under morphine-chloralose anesthesia the cardiac output effects of TEA were much greater. Since vagal tone is high with this anesthetic combination, the initial heart rate of such animals was lower, and TEA produced cardio-acceleration and a parallel increase of cardiac output. The increased cardiac output was accompanied by a fall of venous pressure, and was therefore considered to follow release of the heart from vagal inhibition, rather than contraction of veins or a pri-

mary increase of venous return. Arterial pressure fell, but the decrease of peripheral resistance was much greater than the change predicted by the fall of pressure alone. In all subsequent experiments the animals were anesthetized with thiopental and barbital. The arterial pressure responses to TEA may then be considered to represent chiefly changes of peripheral resistance and hence of vasomotor tone.

It is of interest that in animals under barbiturate anesthesia, heart rate decreased to 112 or less, while in animals under chloralose, heart rate increased to levels of 132 to 150 in response to TEA, although in both cases the heart must have been functionally denervated. It is more than likely that the level of circulating epinephrine is high with the morphine-chloralose combination; a single dose of TEA, by interrupting vagal impulses, would expose the heart to the circulating epinephrine, while continuous infusion, by interrupting the further secretion of epinephrine, would

TABLE I

EXP.	CARDIAC OUTPUT			ART. PRESSURE			PERIPH. RESIST. <sup>1</sup>			HEART RATE		
	Before	After	%Δ	Before	After	%Δ	Before	After	%Δ	Before	After	%Δ
<i>A. Pentothal-Barbital Anesthesia</i>												
2	530	546	+3	117	97	-17	.22	.18	-18	96	80	-17
4	500	500	0	94	68	-28	.19	.14	-28	135	96	-29
6	500	490	-2	90	34	-62	.18	.07	-61	128	98	-23
7	427	470	+10	95	68	-28	.22	.14	-35	114	105	-8
9	450	446	-1	120	70	-42	.27	.16	-41	120	112	-7
<i>B. Morphine-Chloralose Anesthesia</i>												
1	540	810	+50	117	98	-16	.22	.12	-45	75	132	+76
3	607	864	+42	124	83	-33	.20	.10	-50	90	144	+60
5	520	787	+51	158	98	-38	.30	.13	-57	99	150	+51

<sup>1</sup> Roughly estimated as the ratio of pressure in mm. Hg to cardiac output in cc/min.

probably have resulted in a gradual decline of heart rate to levels comparable with those in barbitalized animals.

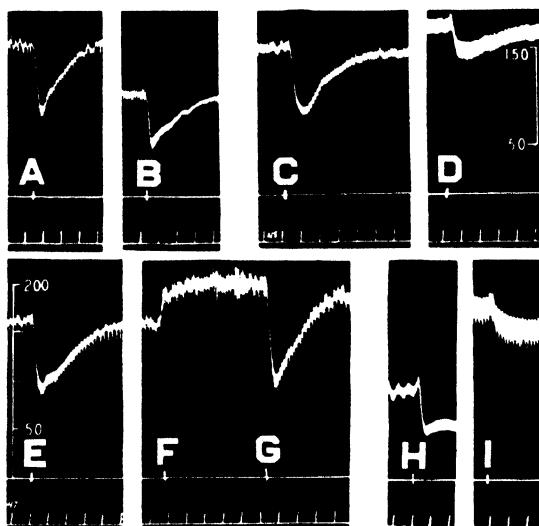
*Buffer Reflexes.* After determining the dose of TEA causing a maximal depressor response, both carotid arteries were clamped. Pressure increased by 20 to 90 mm. Hg in various experiments ('neurogenic' hypertension). The test dose of TEA was then repeated. In all cases, pressure fell to nearly the same floor as during control period, illustrating that the hypertension was indeed neurogenic (fig. 1, EFG). When pressure was elevated to still higher levels by carotid occlusion combined with bilateral vagal block, or by section of all the buffer nerves, TEA again lowered pressure to the original floor. The results suggested that a dose of TEA capable of completely blocking a moderate level of sympathetic outflow is also adequate to block an increased level of sympathetic tone.

To make certain that changes of cardiac output played no role in this response, the procedure was repeated in several of the cardiac output experiments. In one such experiment TEA during the control period reduced arterial pressure by 28 per

cent and total peripheral resistance by 35 per cent, with a 10 per cent increase of cardiac output (*exp.* 7, table 1). After bilateral vagotomy and carotid occlusion, the same dose of TEA lowered the arterial pressure from its elevated level by 53, and decreased peripheral resistance by 49 per cent with an increase of cardiac output of 9 per cent. Similar results were obtained in other experiments. Again the change of pressure induced by TEA reflected with acceptable accuracy the change of peripheral resistance. In contrast to the recent results of Charlier (8), carotid occlusion caused little or no alteration of cardiac output.

As expected, continuous infusion of TEA completely blocked the reflex response to carotid clamping. Depression of the response was significant at an infusion rate of 5 mg/kg/hr., and maximal at 15 mg/kg/hr. At this infusion rate, carotid occlusion raised the pressure no more than clamping of the two femoral arteries.

Fig. 1. SEGMENTS A, B, E, F, G, H, and I: dog, 24.5 kg., anesthetized with thiopental and barbital; segments C and D, dog, 16.2 kg., thiopental and barbital. A and B, responses to maximally depressor dose of TEA. (20 mg.) before and during infusion of glyceryl trinitrate, 3 gamma/kg/min. C and D, responses to TEA (40 mg.) before and during infusion of angiotonin, 0.7 units/kg/min. E and G, responses to TEA before and after occlusion (at F) of the carotid arteries. H, response to TEA after removal of 600 cc. of blood; and I, after rapid reinfusion.



Continuous infusion of TEA also prevented the cardiodecelerator and vasodepressor response to stimulation of Hering's nerve. In these experiments, maximal stimulation of the carotid nerves before administration of TEA produced moderate cardiac slowing, and reduced pressure to exactly the same level as that later produced by TEA itself, indicating that strong stimulation of the carotid receptors may completely abolish vasomotor tone.

Veratridine, which initiates reflex bradycardia, inhibition of vasoconstrictor tone, and apnea (9), failed to produce any effect upon blood pressure during infusion of TEA (10). Since both agents are capable of completely suppressing vasomotor activity, the one by ganglionic blockade and the other by reflex inhibition of the centers, depressor effects of one must obviously be prevented by the other. TEA, by blocking vagal ganglia, also prevented the reflex bradycardia induced by veratridine.

*Epinephrine and Angiotonin.* It has already been demonstrated that TEA does not prevent the vasoconstrictor action of epinephrine; i.e., it is not 'adrenolytic'

(1). In the present study, epinephrine and angiotonin<sup>5</sup> were infused at increasing rates and the response to TEA determined at intervals.

Infusion of epinephrine at low rates (0.1–0.25 gamma/kg/min.) induced a slight fall of arterial pressure, presumably because of its vasodilator activity in low concentration. Under these circumstances TEA caused a fall of pressure to a floor which was lower than normal (fig. 2). Under the vasodilator influence of epinephrine arterial pressure had been maintained by reflex elevation of vasoconstrictor tone. Injection of TEA therefore caused a greater fall of pressure than under control conditions, and the decline in the intrinsic (floor) resistance of the vessels was unmasked by the injection of the blocking agent.

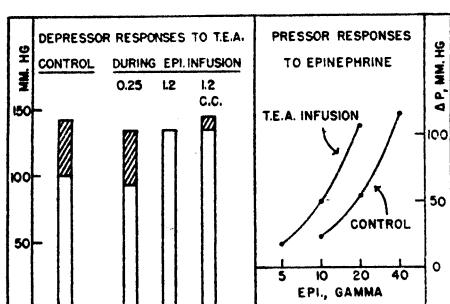
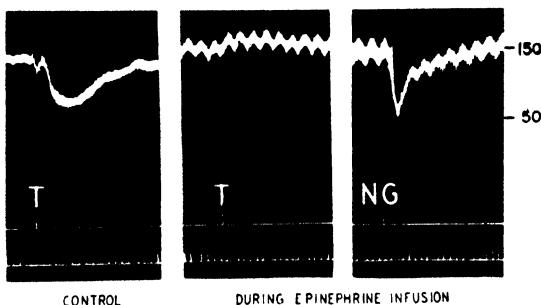


Fig. 2. (Left side) EXPERIMENT 4-13-46. Dog 9.6 kg., anesthesia, thiopental and barbital. Total height of columns, level of arterial pressure immediately before injection of maximally depressor doses of TEA; shaded portion of columns, depressor response to TEA. First column, control; second, during epinephrine infusion at a rate of 0.25 gamma/kg/min.; third and fourth columns, during infusion of epinephrine at 1.2 gamma/kg/min.; fourth column, carotids clamped. (Right side) Exp. 4-25-46, dog 24 kg. Dose response curves to epinephrine (total dose at bottom) before and during continuous infusion of TEA. (20 mg/kg/hr.).

Fig. 3. EXPERIMENT 10-21-46, dog 9.4 kg. At T, TEA, 2 mg/kg., before and during infusion of epinephrine, 0.6 gamma/kg/min. At NG, 0.05 mg/kg. of glyceryl trinitrate.



At higher infusion rates of epinephrine, although arterial pressure still did not exceed control values, the depressor response to TEA was completely lost (figures 2 and 3). It is apparent that the animal had compensated for the constrictor action of epinephrine by reflex inhibition of vasoconstrictor tone (as well as by cardiodeceleration). Compensatory mechanisms are exhausted when vasoconstrictor tone has been completely suppressed, and when this happens, TEA, of course, can cause no decrease of pressure. Frequently TEA caused a slight increase of pressure in the presence of epinephrine, for to whatever extent bradycardia was involved in the compensatory mechanism, it too was abolished by TEA (fig. 3). During infusion of

<sup>5</sup> Generously supplied by the Eli Lilly & Co., Indianapolis, through the courtesy of Dr. K. G. Kohlstaedt.

epinephrine in dogs under morphine-chloralose anesthesia, in which reflex bradycardia was more prominent, TEA caused a greater increase of pressure.

During the infusion of epinephrine at rates which completely inhibited vasoconstrictor tone carotid occlusion caused a return of vasomotor activity and a further elevation of pressure; TEA again lowered the pressure to the levels existing before carotid occlusion (fig. 2). Results with angiotonin were similar, except that low infusion rates did not cause vasodilatation (fig. 1D).

Since the chief mechanism available to combat a pressure rise induced by epinephrine is reflex inhibition of vasomotor tone, it is evident that infusion of TEA will potentiate the pressor effects of small doses of epinephrine. This has already been observed (11, 12). Since the pressure rise begins from the hypotensive level established by TEA infusion, the response in mm. Hg and in percentage of the initial pressure is considerably enhanced (fig. 2); however, the peak levels of pressure reached is often not much higher than that in the absence of TEA, and even these moderate increases can be accounted for by the removal of vagal cardiac inhibition. By reflex means, sufficiently large doses of epinephrine completely abolish vasoconstrictor tone even in the absence of TEA; thus the same level of pressure will be reached as when vasomotor tone was zero initially.

Maximal potentiation of epinephrine occurred at the same infusion rates of TEA which completely prevented the pressor effects of carotid clamping.

*Glyceryl Trinitrate.* Infusion of this vasodilator agent activates compensatory mechanisms, chief among them being increased vasoconstrictor discharge. TEA injected during infusion of glyceryl trinitrate reduced the pressure to a floor which was lower than the control level, for the same reasons indicated above in the discussion of low infusion rates of epinephrine (fig. 1A, B). Similarly, infusion of TEA, by preventing compensatory reflexes, enhanced the depressor action of glyceryl trinitrate.

*Hemorrhage and Pethora.* After withdrawal of small amounts of blood, arterial pressure declined briefly and returned to control levels. Injection of TEA, by abolishing the reflexly increased vasomotor tone, now reduced the pressure to a floor lower than normal just as in the case of infusion of vasodilator agents (fig. 4). After hemorrhage, however, the lower level of pressure reached following injection of TEA must have been the result of diminished cardiac output rather than reduction of intrinsic vascular tone. Withdrawal of more blood caused further reduction of the floor level (fig. 1H and fig. 4). When hemorrhage was of moderate degree, carotid occlusion still caused a rise of pressure, though less than in control periods: vasomotor centers were therefore still not discharging at maximal capacity (fig. 4). When hemorrhage was continued to a more severe degree, the response to carotid occlusion was greatly diminished, and the full vasoconstrictor power available to the animal must have been acting in an attempt to maintain arterial pressure (cf. 13). TEA given at such a time caused a decline to very low levels, and recovery was greatly prolonged.

In two experiments blood was drawn at regular intervals until arterial pressure fell to moderately hypotensive levels, and was then reinjected. The same procedure was repeated during continuous infusion of TEA. In the presence of ganglionic

blockade each withdrawal of blood caused a greater fall of pressure, with a more prolonged and less complete recovery than during the control period.

Plethora caused by rapid infusion of heparinized blood raised arterial pressure by increasing blood volume and cardiac output. In this circumstance the depressor response to TEA was reduced (fig. 11 and fig. 4) suggesting that reflex withdrawal of vasoconstrictor tone had occurred in an effort to prevent the pressure rise. The increased floor did not, of course, represent increased intrinsic vascular tone, but rather reflected the increased cardiac output and blood volume.

*Blockade of Reflexes as Illustrated by Blood Flow Studies.* Injection of moderate doses of epinephrine caused an increase of mean arterial pressure accompanied by a great increase of femoral arterial blood flow. Resistance of the femoral vascular bed was reduced. During continuous infusion of TEA, epinephrine caused a greater pressor response accompanied by a relative reduction of femoral flow; resistance of the femoral bed was greatly increased (12). It is apparent that the vasodilatation

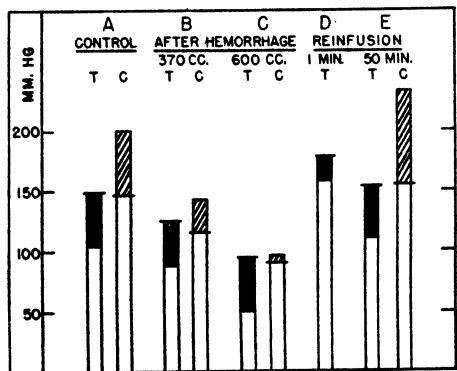


Fig. 4. EXPERIMENT 4-25-46. Depressor responses to TEA (black) and pressor responses to carotid occlusion (diagonal lines). The cross-bar on each column indicates the pressure just before each maneuver. D and E, 1 minute and 50 minutes after reinfusion of all the blood previously removed.

observed in the first instance was reflex in nature; TEA effectively prevented the reflex compensatory mechanism.

In the renal artery, blood flow was diminished by intravenous epinephrine both before and after administration of TEA. The lack of a reflex vasodilator response to epinephrine in the kidney does not imply a more intense constrictor action in the renal as opposed to the femoral circulation, but suggests that in the anesthetized dog the renal arteries are subjected to relatively little vasoconstrictor tone and therefore cannot partake in the compensatory vasodilator effort. This is confirmed by the response of renal blood flow to TEA alone. During the fall of arterial pressure induced by maximally depressor doses of TEA, renal blood flow was either unchanged or diminished, although femoral flow was considerably increased.

Accompanying the fall of pressure induced by injection of glyceryl trinitrate there was, after an initial brief increase, a diminution of femoral blood flow due to reflex vasoconstriction which persisted even after blood pressure returned to normal. During infusion of TEA, glyceryl trinitrate produced an increased femoral blood flow even though arterial pressure fell to lower levels than before (12).

Reflex vasoconstriction induced by vasodilator agents also occurred in the kidney. Injection of histamine caused a fall of arterial pressure accompanied by a decrease of renal blood flow. The diminished renal flow reflected in part the fall of perfusion pressure, but since flow recovered less rapidly than pressure, it is obvious that the renal vessels were also involved in the reflex vasoconstrictor effort. Thus it is seen that the renal vessels respond very little in compensatory mechanisms excited by pressor agents, but can partake in the compensation to depressor drugs.

*Asphyxia.* Although asphyxia is believed to cause intense activity of the sympathico-adrenal system, TEA prevented neither the asphyxial nor the post-asphyxial pressure rise (14). No interpretation of this anomalous response can be formulated until further studies have been completed.

#### DISCUSSION

These experiments indicate that in animals under barbiturate anesthesia the depressor response to TEA provides an adequate estimate of the neurogenic component of peripheral resistance. Procedures expected to increase sympathetic pressor outflow (carotid occlusion, section of buffer nerves, hemorrhage, and infusion of vasodilator drugs) enhanced the relative depressor response to TEA; procedures which reduced sympathetic outflow (plethora, infusion of epinephrine) diminished the relative depressor response to TEA. Cardiac output changes induced by the drug were slight under the conditions of these experiments. In the experiments of Eckenhoff *et al.* (15) cardiac output was significantly lower after TEA than in control observations. However, approximately an hour had elapsed between control and test procedures, and the cardiac output of control animals which had received no TEA was also diminished, confirming the report of Shore *et al.* (16) that cardiac output decreases considerably with time in animals under barbital anesthesia.

It should be recognized that under conditions of severe stress when adequate arterial pressure is maintained only by nearly maximal discharge of pressor mechanisms (hemorrhage, nitrite shock), arteriolar dilatation induced by TEA may cause a sufficient further pooling of blood to reduce venous return and hence cardiac output. Reduced cardiac output may account for occasional cases of 'circulatory collapse' reported when the drug is administered to patients with severe hypertension (17).

In human subjects measurements of cardiac output by means of the ballistocardiograph and the Fick method indicate that TEA usually causes a moderate increase, rarely a decrease, of cardiac output (5, 18). As a rule, the depressor response to TEA must underestimate the true degree of vasomotor tone. Since in the human subject in whom vagal tone is high the heart rate is increased by TEA, these results are comparable in direction if not in magnitude with those obtained in dogs under chloralose anesthesia in the present study.

The vasodepressor effects of carotid sinus nerve stimulation were completely abolished by TEA. If the depressor reflex involves excitation of vasodilator mechanisms as well as inhibition of vasoconstrictor tone (19), then it must be concluded that the vasodilator paths are also blocked by TEA. However, if vasodilator discharge is of importance in the carotid reflex, maximal stimulation of the carotid sinus nerves

should reduce arterial pressure more than does TEA itself. This was not found to occur. Furthermore, if vasodilator mechanisms take part in regulation of arterial pressure, they should be invoked as part of the compensatory effort aroused by infusion of epinephrine. That is, reflex inhibition of vasoconstrictor tone and reflex increase of vasodilator tone should *both* occur. If this be true, TEA should produce an increase of arterial pressure of considerable magnitude during infusion of epinephrine. The observed pressor responses to TEA were slight, and could be adequately accounted for by cardioacceleration (blockade of reflex vagal effects). The failure of TEA to provoke more than a slight elevation of pressure during infusion of epinephrine indicates either that activation of vasodilator mechanisms does not occur as part of the compensatory effort, or that whatever vasodilator paths are involved cannot be blocked by TEA (as would be true if such paths were non-ganglionated). It has frequently been suggested that vasodilator pathways leave the spinal cord through the dorsal roots. Such unique channels might not be interrupted by ganglionic blocking agents. However, Berenthal *et al.* (20) have demonstrated that extensive sympathectomy completely prevents carotid body vasomotor reflexes. Their evidence supports the conclusion that barostatic reflexes are mediated chiefly if not entirely by modulation of vasoconstrictor activity (including, of course, veno-motor mechanisms).

Reactions of the renal and femoral vascular areas to epinephrine, vasodilator drugs, and TEA show, at least in the dog under barbital anesthesia, that vasomotor tone is relatively low in the kidneys, and high in somatic areas. Compensatory vasodilatation in response to pressor agents can occur only in areas which are initially subject to vasoconstrictor tone; i.e., femoral and presumably other somatic vessels. Compensatory vasoconstriction can and doubtless does involve both somatic and visceral vascular beds. The evidence available shows that human buffer mechanisms may to some extent resemble those in the dog. TEA causes little change of renal blood flow, but usually a well-marked increase of flow in the hands and feet. Glycerol trinitrate and other agents which relax smooth muscle may lower pressure, but rarely increase blood flow in the extremities; the direct action of the drug is overcome by reflex constriction. For this reason only agents which block vasoconstrictor pathways at some point may be expected to cause useful hyperemia of the extremities.

#### SUMMARY

The depressor action of TEA in barbitalized dogs is due to reduction of peripheral resistance. In dogs under chloralose the reduction of peripheral resistance may be masked in part by increased cardiac output. The response to TEA may be used to estimate the degree of vasoconstrictor tone existing during various experimental conditions; for example, during neurogenic hypertension, infusion of pressor or depressor drugs, hemorrhage, and plethora. By interrupting compensatory reflexes, TEA potentiates pressor and depressor drugs. By interrupting the efferent pathways, TEA prevents the cardiovascular effects of veratridine. Injection of histamine induces compensatory vasoconstriction in both the femoral and renal vascular beds; epinephrine induces compensatory vasodilatation in the femoral but not the renal

circulation. The compensatory effort induced by injection of pressor drugs appears to be accomplished mainly by inhibition of vasoconstrictor discharge; increased vasodilator activity could not be demonstrated.

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# HEMODYNAMICS OF AORTIC OCCLUSION<sup>1</sup>

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AS EARLY as 1886, de Jager (1) found that occlusion of the aorta directly above the diaphragm causes a considerable increase in the carotid pressure. Later, other authors observed blood pressure rises after clamping of large arteries and, more recently, blood pressure changes after aortic clamping were studied by Barcroft (2), by Brotchner (3) and by Hamilton and Remington (4). Most of the authors mentioned recognized the blood pressure rise as a hemodynamic phenomenon which is independent of any of the neural (1-3) or humoral (3) mechanisms which regulate the blood pressure. Barcroft made the surprising observation that occlusion of the aorta results in an increase of the cardiac output. In the present study some of these experiments have been repeated and expanded, and an attempt has been made to devise a model of the circulation which accounts for the mechanisms producing the phenomena observed.

## METHODS

Since a study of hemodynamic phenomena was contemplated, all experiments were carried out on vagotomized animals in deep Nembutal narcosis, in order to depress the circulatory reflexes as much as possible without reducing the circulation to a shock level. Blood pressure was recorded with a mercury manometer connected with one of the carotids. The aorta was occluded by a loop of thick cotton string placed around this vessel between the diaphragm and the celiac artery. In some experiments the same procedure was used for the occlusion of aortic branches. Cats were used in all experiments.

## RESULTS

Figure I, I shows the blood pressure changes in a deeply narcotized and vagotomized animal when the aorta is clamped directly under the diaphragm. The general course of the pressure rise is asymptotic. The presence of the neural regulating mechanisms of heart and vessels can disturb this course as shown in figure I, II. In this preparation the vagus nerves were not severed. The heart rate was 164 before clamping the aorta. Due to the depressor and carotid sinus reflexes the rate dropped during the pressure rise to 148, to increase again after release of the clamp to 169. The record shows that the blood pressure, after reaching a maximum,

Received for publication December 10, 1948.

<sup>1</sup> Aided by a grant from the U. S. Public Health Service.

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started to fall even though the clamp on the aorta was left in place. After release of the clamp the pressure dropped considerably below the original level to recover only slowly. In preparations in which narcosis was light these features were even more pronounced. Indications of these deviations from the asymptotic curve are occasionally seen in narcotized and vagotomized preparations and are probably due to residuals of the regulatory activity transmitted through the undisturbed part of the autonomic system. In preparations not showing this activity, the increased blood pressure is maintained during occlusion of the aorta for 5 to 10 minutes; when the clamp is then removed, the pressure drops asymptotically. The occlusion of smaller arteries has a similar but smaller effect on the blood pressure as clamping the aorta. This effect is cumulative as is shown in figure 1, III in which successively the celiac and the superior mesenteric arteries were clamped.



Fig. 1. EFFECT OF AORTIC OCCLUSION in a vagotomized and deeply narcotized preparation (I). The same in a preparation with intact vagi (II). Occlusion, first of the celiac then of the superior mesenteric artery, followed by a release of both arteries together (III). In this and in fig. 2 and 3 an arrow pointing down indicates occlusion, one pointing up release of the vessel. Calibration is from 9 to 16 cm. in I, from 7 to 14 cm. in II and from 8 to 14 cm. in III. Time in sec.

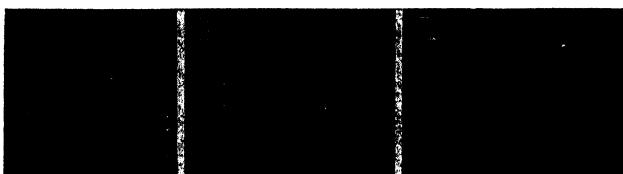


Fig. 2. EFFECT OF POSITION of the preparation on the blood pressure reaction due to aortic occlusion. I, the animal is in the horizontal position; II, tilted with the head up, and III, tilted with the head down. Calibration from 8 to 18 cm. Time in sec.

In support of de Jager's, Barcroft's, and Brotchner's concept of the effect of arterial occlusion as a hemodynamic, rather than a reflex phenomenon, it was found that destruction of the spinal cord, or asphyxiation for 20 minutes of the entire central nervous system by raising the intradural pressure above the arterial pressure (5), did not abolish the reaction on aortic occlusion, even though these procedures reduced the blood pressure to a few cm. Hg. Also the injection of nicotine in doses large enough to stop conduction in the sympathetic ganglia (100-200 mg/kg. body-weight) had little effect on this phenomenon.

De Jager first came to the conclusion that after clamping the aorta blood flows cephalic from the occluded area. The importance of this flow for the blood pressure rise after clamping can be demonstrated in the following way. Tilting the animal with the head down will facilitate the flow of blood out of the inferior vena cava region, tilting with the head up will hamper that transport. Figure 2 shows that

the blood pressure response on clamping the aorta is indeed modified by tilting the animal. The response with the head up is smaller, with the head down larger than the response in the level animal. Another way of showing the importance of the transport out of the inferior vena cava region for the blood pressure response is shown in figure 3. The first pressure changes in this figure (I and II) are the usual effects of clamping and releasing the aorta. At III (fig. 3) the vena cava is occluded directly under the diaphragm. This causes a drop in blood pressure which is due, as recognized by de Jager, to a loss of blood through the aorta into the lower part of the animal. A few seconds later (IV) the aorta is clamped causing only a small rise of the blood pressure. After release of the vena cava (V), however, a blood pressure rise develops which resembles the increase of figure 3, I in which the aorta was

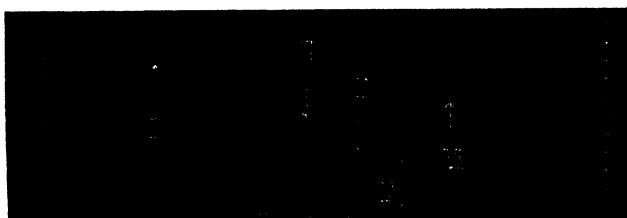


Fig. 3. EFFECTS OF CLAMPING THE AORTA and inferior vena cava. I and II, aortic occlusion and release; III, clamping of the vena cava; IV, aortic occlusion; V, release of the vena cava; VI, release of the aorta. Calibration from 7 to 16 cm. Time in seconds.

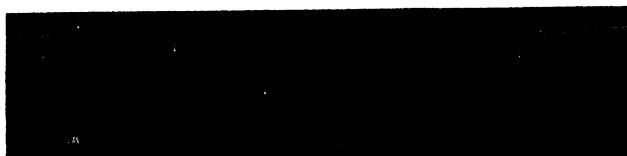


Fig. 4. EFFECT OF REMOVAL and injection of blood. At each of the arrows pointing down, 10 cc. blood is removed, at each of the arrows pointing up 10 cc. blood is reinjected. Calibration from 3 to 17 cm. The block indicates 10 seconds.

clamped while the vena cava was patent. The force which moves the blood out of the arteries and veins of the occluded area is the elasticity of the vessels. As long as the blood flow is unimpaired the vessels are extended by the pressures which cause this flow. After clamping the aorta the pressures in the occluded area become equal to that in the right atrium, causing a retraction of the vessels, which force their contents into the cephalic part of the circulation.

The filling of the circulatory system was found to affect the blood pressure markedly in deeply narcotized and vagotomized preparations. Figure 4 shows an experiment in which 10 cc. blood is withdrawn 3 times from the circulation in such a preparation. Each time this causes a permanent drop in the pressure. When the blood is injected again 10 cc. at the time, the pressure returns to the original level in three steps. In more reactive preparations the circulatory reflexes compensate for the effects of such relatively small changes in blood volume.

## A MODEL OF THE CIRCULATION

A model including the pertinent features of the circulation is schematized in figure 5. It is assumed that the pump has that characteristic of the heart which makes the stroke volume dependent on the amount of fluid flowing into the pump between two strokes (Starling's law). The connections between the out- and inlet side of the pump consist of rigid tubing, and the elastic properties of the vascular system on the arterial and venous side are represented by the capacitors  $C_1$  and  $C_2$ . The peripheral resistance (arterioles and capillaries) is represented by the resistance to flow,  $R$ . The resistance  $r$  represents certain properties of the pump and also includes the resistance in the venous system.

The steady state relations among the pressures on the outlet and inlet side and the flow in the system on the one hand, and the various parameters of the circuit on the other can be found as follows. The difference between the environmental pressure and the pressure at the outlet side is designated as  $P_1$ , the pressure between the former and the pressure in the region of the system between the resistors  $R$  and

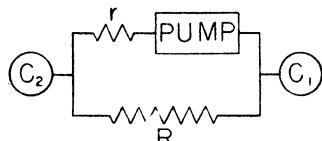


FIG. 5. CIRCULATORY MODEL (for explanation see text)

$r$ , as  $P_2$ . The pressure drop for viscous flow through the resistor  $R$ , assuming that its properties are independent of pressure,<sup>4</sup> will be proportional to the rate of flow,  $I$

$$P_1 - P_2 = I R \quad (1)$$

where  $R$  includes the viscosity of the fluid.

The pump is considered as operating at constant stroke frequency, with connection alternately to the input and output sides. In filling it will operate as an elastic vessel, expanding to a volume which is proportional to the input pressure. Thus the mean rate of flow through the pump will be proportional to the input pressure<sup>5</sup>

$$I = P_2 / r \quad (2)$$

All additional parameters limiting the flow into the pump are lumped in the constant  $r$ , including input resistance, inertia, the time restriction of filling according

<sup>4</sup> In reality the pressure-flow relation is not linear (6, 7). However in the normal pressure region the deviation from linear relationship is negligible.

<sup>5</sup> It follows from the work of Patterson, Piper and Starling (8) that an increase of the heart output at constant arterial pressure is coupled with a dilatation of the heart and an increase in venous pressure, which is necessary to increase the stroke volume and also to dilate the heart. Since both of these factors causing the increase in venous pressure will be approximately proportional to flow, equation (2) will describe these conditions adequately. It follows further from Patterson, Piper and Starling's work that an increase of the arterial pressure at constant heart output is also coupled with heart dilatation and increased venous pressure. This effect of arterial on venous pressure is not represented in (2). This is probably permissible with a heart in good condition (no large systolic residue); in a failing heart, however, this simplification may become increasingly serious.

to the length of the pump cycle, etc. It will be assumed that  $r$  is constant for a given stroke frequency.

As the pressure is increased on the inlet or outlet side the capacitors will contain correspondingly larger volumes of fluid. It will be assumed that  $V$ , the increase in fluid volume in a capacitor is directly proportional to the gauge pressure,<sup>6</sup> such that for the output and input capacitors

$$\begin{aligned} V_1 &= C_1 P_1 \\ V_2 &= C_2 P_2 \end{aligned} \quad (3)$$

where  $C_1$  and  $C_2$  are constants of capacitance for the capacitors (volume increase per unit change of pressure). For a constant total volume of fluid in the system the sum of  $V_1$  and  $V_2$ , which represents the volume of fluid transferable from one capacitor to the other, must also be constant

$$V_1 + V_2 = V \quad (4)$$

Eliminating  $V_1$  between (1) and (2)

$$(P_1 - P_2)/R = P_2/r \quad (5)$$

Substituting (3) in (4)

$$C_1 P_1 + C_2 P_2 = V \quad (6)$$

Eliminating  $P_2$  between (5) and (6) yields for the pressure on the outlet side

$$P_1 = \frac{V(R + r)}{C_1 R + (C_1 + C_2)r} \quad (7)$$

Elimination of  $P_1$  between (5) and (6) gives for the pressure on the inlet side

$$P_2 = \frac{Vr}{C_1 R + (C_1 + C_2)r} \quad (8)$$

From (2)

$$I = \frac{V}{C_1 R + (C_1 + C_2)r} \quad (9)$$

Some consequences are immediately obvious. Both pressures at outlet (arterial) and inlet (venous) side as well as flow (minute volume of the heart) increase with the volume of circulating fluid. An increase in peripheral resistance ( $R$ ) reduces pressure on the inlet side and flow but increases the pressure on the outlet side. However, since  $R$  is present in the numerator as well as in the denominator of (7), whereas it is present only in the denominator of (8) and (9), it has to be expected that the relative effect of a change in  $R$  is considerably less on the pressure at the outlet side than on the flow and on the pressure at the inlet side. This is because an increase in peripheral resistance depresses the flow in the system and thus counteracts the effect of the higher resistance on the pressure at the outlet side. Increase of  $C_1$  and  $C_2$  individually or together will result in a decrease of the pressure both at

<sup>6</sup> We are not aware of studies in which the filling of a vascular area is related to the pressures prevailing in its parts (arteries, arterioles, capillaries and veins) under normal conditions. The pressure-volume relation in the aorta (7), however, is almost linear. In the vena cava inferior this relation deviates from linearity especially in the region of the lowest pressures.

the outlet and inlet side and of flow. Such a change would be caused by a loss of tone in the arterial and venous system of the animal.

#### DISCUSSION

If the equations describing the steady state relations in the model are to represent the blood circulation, there must eventually be conformity between the observed circulatory phenomena in the animal experiments and the predictions which can be made from the equations after the introduction of appropriate parameters to make them fit individual conditions.

Marked and permanent decreases and increases of the arterial pressure were observed after removal of blood from, or injection of fluid into the circulatory system of the cat. Since such changes in  $V$  are directly proportional to the arterial pressure (7) this is in agreement with the predictions made on the basis of a study of the circulatory model.

In the model the constant of capacitance is independent of the peripheral resistance ( $R$ ). In the animal experiment clamping the aorta not only increases the peripheral resistance, but also eliminates part of the arterial and venous capacitors. This is obvious for the arterial capacitor but since the vena cava is in wide connection with the right atrium where the pressure is low, the venous capacitor in the caudal part of the body is functionally eliminated even if the vena cava inferior is not actually clamped. If large branches of the systemic arterial tree are clamped, it is reasonable to assume that the changes in the arterial and venous capacitances are inversely proportional to  $R$ , since the capacitors are formed by the vessels themselves and the larger the part of the vascular system which is occluded the greater its capacitance, and also the greater its conductance  $\left(\frac{1}{R}\right)$ . The constants of capacitance for the arterial and venous systems therefore can be written under those circumstances as  $\frac{C'_1}{R}$  and  $\frac{C'_2}{R}$ . Substituting this in (7), (8) and (9) gives:

$$P_1 = \frac{RV(R + r)}{C'_1 R + (C'_1 + C'_2)r} \quad (10)$$

$$P_2 = \frac{RVr}{C'_1 R + (C'_1 + C'_2)r} \quad (11)$$

$$I = \frac{RV}{C'_1 R + (C'_1 + C'_2)r} \quad (12)$$

There is evidence that after clamping the aorta the arterial and venous capacitors in the caudal part of the animal empty into the right atrium where the average pressure is near zero. The fraction of  $V$  situated in the caudal part of the preparation thus is shifted into the remaining part of the circulation and the transferable amount of blood ( $V$ ) in the reduced arterial and venous capacitors is the same as that in the larger capacitors before aortic clamping. Since after establishment of the new equilibrium following aortic clamping,  $V$  remains practically unchanged and  $R$  is greatly enlarged, an increase can be expected of the arterial and venous pressure and of the blood flow in the cephalic part of the circulation. As  $R$  appears in the nu-

numerator and in the denominator of (11) and (12), the effect on  $P_2$  and I will be small as compared with the change of R. However, since the change in R due to aortic clamping is so large, an easily measurable increase in the venous pressure and in the blood flow can be predicted. In (10) R appears to the second power in the numerator and only to the first in the denominator and thus a relatively greater effect on the arterial pressure is to be expected. It seems surprising that occlusion of a large part of the circulation would increase the flow through the heart. However, this paradoxical effect of aortic clamping has been observed. Barcroft found that occlusion of the aorta directly under the diaphragm in vagotomized preparations caused regularly an increase of the heart output up to 53 per cent (the average increase in 21 experiments was 25 per cent). The same phenomenon was observed in more than half of 26 preparations in which all reflex activity was prevented by asphyxial destruction of the brain, thus showing it to be of hemodynamic origin. In the rest of these 26 preparations aortic occlusion caused either no change of the heart output or a slight decrease. It has been remarked above, that the use of equation (2) is probably only justified when the heart is in good condition. The low arterial pressure produced by destruction of the brain may well have an averse effect on the oxygenation of heart, and this may explain the variability of the effect of aortic occlusion in such preparations.

Barcroft as well as Hamilton and Remington (4) found, that in preparations with intact vagus nerves, aortic occlusion often fails to cause an increase in heart output. This is probably due to the activity of the circulatory reflexes, since in the data of Hamilton and Remington the preparation showing the greater reduction of the heart output during occlusion, also showed the greater decrease of the heart rate in this period.

De Jager observed during aortic occlusion the expected increase in the (jugular) venous pressure.

The relation between the change in R and the change in arterial and venous capacitances due to vascular occlusion will differ for the various circulatory areas. In the splanchnic region, for instance, with its very large venous capacitor the decrease in capacitance of the venous system caused by clamping the celiac and mesenteric arteries will undoubtedly be larger than indicated by the relation  $\frac{C'_2}{R}$ ;

in the renal circulation which has only a limited capacitance the decrease in the capacitances of the arterial and venous system will probably be smaller than indicated by that relation. The increase in blood flow will therefore in the first instance be larger than indicated by (12); in the case of the renal circulation the increase in flow will be smaller than would follow from that equation, or flow might even be decreased as compared with that before clamping. In clamping a very large part of the circulation including many vascular areas (as in aortic occlusion), it is more likely that the changes in the capacitances and the change in R indeed approach inverse proportionality.

In the experiment in which the aorta was clamped while the vena cava inferior was occluded, the transport of blood from the caudal part of the animal was prevented. The amount of transferable blood (V) in the reduced circulation thus is

smaller than in the larger circulation before aortic clamping. The reduced blood volume will be roughly inversely proportional to  $R$ , if we assume that the amount of blood in a vascular area is proportional to its size and thus to its conductivity  $\left(\frac{1}{R}\right)$ . The transferable volume thus can be written as  $\frac{V}{R}$ . The equations (11), (12) and (13) then revert to (7), (8) and (9). It would follow from the latter that a rise in arterial pressure is to be expected, which is small with respect to the change in peripheral resistance and relatively larger drops in venous pressure and flow. These predicted changes in arterial pressure and flow have been observed by Barcroft (2). Also in the present experiments the pressure rise due to aortic clamping was greatly reduced by occlusion of the vena cava inferior (fig. 3). A complication not considered until now is present in the capacitors formed by the vessels of the lung circulation and also by the heart. These capacitors are small as compared with the large arterial and venous capacitors of the systemic circulation, and their neglect when considering the normal circulation seems justified. However in a reduced circulation as after occlusion of the aorta and vena cava inferior it is advisable to take their effect into account. The reduction of the heart output due to the clamping of these two vessels observed by Barcroft, may result in an emptying of the lung and heart capacitors into the reduced systemic circulation, increasing its blood volume and thus causing a larger rise in arterial pressure and a smaller decrease of venous pressure and of flow than would follow from the equations (7), (8) and (9). Barcroft believed this transfer of blood from the lung circulation and also from the heart into the reduced systemic circulation to be the sole cause of the rise in arterial pressure after simultaneous occlusion of aorta and vena cava inferior. However, it follows from (7) that even without these capacitors a small rise in arterial pressure is to be expected.

It has been possible to explain with the equations (7), (8) and (9) and a few reasonable assumptions, a number of observations on the blood circulation and it therefore seems allowable to consider these equations as a first approximation of a mathematical description of the circulation. It is by changing the values of the various parameters that the circulatory reflexes regulate the circulation. For instance, the main effect of an arteriolar contraction is an increase of the peripheral resistance, but this will also affect the capacitance of the system. Increase in tone of arteries and veins affects mainly the constants of capacitance, although the peripheral resistance also may undergo changes. Changes in heart rate will affect  $r$ . A marked slowing of the rate will increase  $r$ , as the pericardium in the intact animal limits the increase in filling compensating for the decreased heart rate (4).

From equations (7), (8) and (9) the conclusion can be drawn that the arterial and venous pressure and the blood flow are equally good indicators for changes in the blood volume filling the capacitors, and for changes in the constants of capacitance of the latter, since there exist relatively simple direct and inverse relations among these parameters. For the same reason the venous pressure and the flow are good indicators for changes in peripheral resistance. The great difference between arterial and venous pressure shows that the peripheral resistance ( $R$ ) is large with respect to  $r$  (see equations 1 and 2), and it follows from (7) in which  $R$  is present

in both numerator and denominator, that under such circumstances changes in the peripheral resistance can have only a relatively small effect on the arterial pressure. Peripheral resistance therefore should be regarded as a regulator of blood flow rather than as a primary determinant of blood pressure. These conclusions make it highly unlikely that the considerable changes in systemic arterial pressure due to activity of the regulatory mechanisms of the circulation are primarily attributable to changes in the peripheral resistance as is usually assumed. Indeed, besides changes in  $R$ , changes in other parameters much more directly related to the arterial pressure are involved in vascular reactions. For instance a generalized sympathetic activity or a release of adrenalin may cause besides 1) contraction of the arterioles, mainly increasing  $R$ ; 2) contraction of the spleen, increasing  $V$ ; 3) increase of tone in the arteries and veins, decreasing the values of  $C_1$  but probably even more of  $C_2$ ; 4) increase of the heart rate which will usually result in a decrease of  $r$ . The first 3 of these factors tend to increase the systemic arterial pressure (7). The changes in  $V$ ,  $C_1$ ,  $C_2$  and  $r$  tend also to increase the flow in the system but the change in  $R$  has an opposite effect. Depending on the numerical values of the parameters involved, an adrenalin injection therefore may or may not increase the total blood flow. It is of interest to note in this respect that Tigerstedt (9) observed during stimulation of the splanchnic nerve, which produces most of the changes mentioned above, an increase of the total flow in the systemic circulation.

#### SUMMARY

A model of the blood circulation was proposed and conclusions drawn from its mathematical description were found to be in agreement with observed effects of aortic occlusion. The relations among arterial and venous pressure and heart output on the one hand and blood volume, peripheral resistance, constants of capacitance of the arterial and venous system and the factor limiting the flow into the heart on the other follow from the description of the model. The conclusion is drawn that peripheral resistance is to be considered mainly as a regulator of blood flow rather than as a primary determinant of arterial blood pressure.

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# THE AMERICAN JOURNAL OF PHYSIOLOGY

*Published by*  
THE AMERICAN PHYSIOLOGICAL SOCIETY

VOLUME 157

May 1, 1949

NUMBER 2

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## LITHIUM: ITS EFFECT ON HUMAN SPERMATOZOA, RAT TESTICULAR TISSUE AND UPON RATS IN VIVO

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DURING an investigation of phosphatase activity in human spermatozoa (1), we discovered, quite accidentally, that the lithium ion, in relatively low concentration, has an inhibiting effect on the metabolism and motility of human spermatozoa (2). The results to be reported here indicate that lithium is worthy of more intensive study, particularly in view of its recent prominence in therapeutics and the knowledge that, in certain circumstances, it may be highly toxic (3).

Lithium has received relatively little attention from physiologists and pharmacologists as evidenced by the sparsity of references in the respective fields. The embryological literature, however, as reviewed by Needham (4) contains many studies which show that lithium has profound effects on the early embryo, particularly in producing exogastrulation and failure of development of the notochord. The nature of its action remains highly obscure. Indeed, Needham (4) in summarizing his review of the effects of lithium states "that up to the present time (1944) a biochemical effect of lithium equivalent in magnitude to its morphological effect has been sought for in vain."

In the pharmacological field, an experimental study of lithium toxicity in cats (5) showed that the chloride (60 mg./kg.) caused extreme muscular weakness, severe gastro-intestinal symptoms and death in from 24 hours to 3 days. In the human being (6) a total of 8 gm. of lithium chloride taken over a period of 28 hours produced marked muscular and general prostration, vertigo and eye and ear symptoms over a period of several days but no gastro-intestinal symptoms. Its absorption and excretion in the human subject has been studied (7) and while no toxic effects were reported, it was shown that soluble Li<sup>+</sup> salts added to a diet are readily absorbed and quantitatively excreted in the urine. It is not absorbed well from a natural diet.

More recently, a commercial preparation<sup>3</sup> containing 25 per cent LiCl has been used widely as

Received for publication February 28, 1949.

<sup>1</sup> This work was done under a research grant from Mr. C. V. Whitney.

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<sup>3</sup> This product has since been withdrawn from the market.

a salt substitute in low sodium diets. A report (3) in publication indicates that, in certain circumstances, LiCl produces hyperirritability and pronounced muscular weakness. One death has been attributed to its use, the patient in question exhibiting the above symptoms prior to coma and death.

There are few references to the effect of lithium on tissue metabolism. The studies on embryos (4) were concentrated on respiratory metabolism with no definite results reported. The growth of yeast and yeast fermentation (8) are not appreciably affected by the ion though other ions in the same series (K, Cs, Rb) exert a considerable stimulating effect on yeast growth. Recently, Ponder (9) has shown that human red cells are most fragile in LiCl. In the field of tissue metabolism, there are references (10) to the effect of various cations on glycolysis and respiration but so far as can be determined, no studies on Li<sup>+</sup> have been done.

The following experiments are confined to the effect of Li<sup>+</sup> on the metabolic and motile behavior of human spermatozoa, its effect on the metabolism of rat testicular tissue and the acute and chronic effects of subcutaneous injection of LiCl in rats.

#### METHODS

The aerobic and anaerobic lactic acid production and respiration of the tissues were measured by the Warburg technique over a period of several hours, at 38°C. by methods already described in detail (11). It should be emphasized that the spermatozoa, in all experiments, were removed from the seminal fluid by centrifugation and re-suspended in Ringer-glucose-bicarbonate for the glycolysis determinations and in Ringer-glucose-phosphate for measurement of oxygen consumption. The testicular tissue was obtained from rats killed by a blow on the head. Tissue aliquots of approximately 150 mg. were weighed on a torsion balance and transferred to Warburg manometer vessels containing measured amounts of the various Ringer glucose solutions. The lithium (in the form of LiCl) was added to the spermatozoa suspension and the testicular tissue from the side-arms of the manometer vessels after equilibration at 38°C. with the appropriate gas mixtures and just prior to the first (zero time) manometric reading. Manometric lactic acid production was checked chemically, in certain experiments, by the Barker and Summerson technique (12).

The *in vivo* experiments were conducted on mature male rats (average weight 250 gm.), maintained on the normal laboratory diet. The LiCl was injected subcutaneously and the rats left under constant observation subsequently.

#### EXPERIMENTS

The spermatozoa were obtained from normal human semen by centrifugation and re-suspended in the appropriate Ringer-glucose solutions for measurements of oxygen consumption, aerobic and anaerobic glycolysis. The standard cell suspension volume used was 1 cc. per Warburg vessel and the total spermatozoon count ranged from 100 to 150 million cells/cc. The motile activity of the cells and the percentage of cells active was determined prior to equilibration of the vessels with the appropriate gas mixtures. The LiCl was placed in the side-arm of the vessels and added to the main vessel containing the cell suspension at the conclusion of the gas equilibration and about 5 minutes previous to the zero manometric reading. Thereafter, readings were taken at 30-minute intervals and the usual duration of each experiment was 3 to 4 hours. Two control suspensions were run in every

experiment and motile examinations were made in these and in the experimental suspensions at the end of each run. Figure 1 shows the effects upon the anaerobic and aerobic lactic production and motile activity of the cells in the presence of the designated molarities of LiCl.

The curves are representative of both aerobic and anaerobic glycolysis since, as shown previously (13), there is virtually no quantitative difference in the two glycolytic mechanisms. The molarities indicated are final molarities in the system.

It will be seen that the inhibition of glycolysis appears rapidly in each dilution used but that in any one concentration, the glycolysis thereafter proceeds steadily at a new, lower steady state rather than undergoing increasing inhibition.

In the highest  $\text{Li}^+$  concentration indicated ( $0.025\text{M}$ ) the total reduction in lactic acid production over the 4-hour period is 40 per cent and this inhibition is remarkably constant in any given experiment using the same  $\text{Li}^+$  concentration. However, two- or three-fold increases in the  $\text{Li}^+$  concentration above this level do not produce progressively greater inhibitions. The highest inhibition attained was 60 per cent at a concentration of  $0.10\text{M}$ . However, complete failure of the motile activity of the spermatozoa (fig. 1) is attained at relatively low concentrations of the ion. As would be expected, there is good correlation between the inhibition of glycolysis and the failure of motility, though it will be evident that a considerable glycolytic acitivity remains when virtually all motile activity has ceased.

*Effect of LiCl on oxygen consumption of spermatozoa.* In contrast to the effect of lithium on glycolysis, similar concentrations of the ion have no effect on the oxygen consumption. In a series of experiments using concentrations of  $\text{Li}^+$  which would depress glycolysis about 50 per cent, no striking effect on  $\text{O}_2$  consumption could be observed.

*Effect on metabolism of rat testicular tissue.* Experiments were designed 1) to determine if similar effects of the ion could be produced in testicular tissue, and 2) if lithium affected the production of normal spermatozoa in the mature animal. For obvious reasons, it is not possible readily to obtain normal human testicular tissue. Furthermore, it was not considered advisable to risk human *in vivo* experiments. The rat was selected as being the most appropriate experimental animal, since breeding experiments can be performed within a short space of time and the metabolic activity of the testicular tissue of that animal is known. One of the characteristics of rat testicular tissue is a considerable production of lactic acid under aerobic conditions.

The effect of lithium on the rat testicular tissue is summarized in table 1. The concentrations of the ion inhibiting the glycolysis and motility of human spermatozoa have no detectable effect on the respiration and anaerobic glycolysis of testicular tissue but do have definite stimulating effect on aerobic glycolysis. The experiments recorded in the table were measured manometrically, but chemical checks on the lactic acid production were made in certain experiments to be certain that the extra acid produced was lactic acid. Though perfect chemical checks were not obtained, it is certain that at least 85 per cent of the extra aerobic acid produced in the presence of lithium is lactic acid. These results are in distinct contrast to the inhibiting effect of the ion on the glycolysis of spermatozoa.

Up to isotonic concentrations of lithium only aerobic glycolysis is affected, the highest elevation of the latter in this range being about 75 per cent. On the hypertonic side (0.2M), the ion begins to produce a slight depression of oxygen consumption, though relatively high concentrations inhibit respiration to a considerable degree. Anaerobic glycolysis is not affected appreciably in any concentration tested. These findings are of more than ordinary interest and will be discussed later.

*Effect of lithium on rats in vivo.* As a further extension of the investigations of Li<sup>+</sup> on the germinal tissues, LiCl was injected subcutaneously into mature male rats to determine 1) the acute effects of the ion and 2) if spermatogenesis was af-

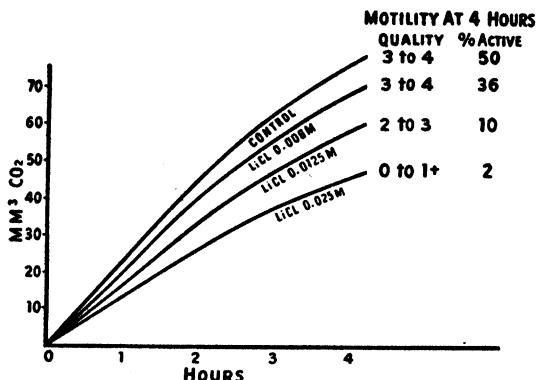


Fig. 1. EFFECT OF LITHIUM  
on motility and lactic acid production  
of human spermatozoa.

TABLE I. EFFECT OF LiCl ON METABOLISM OF RAT TESTICULAR TISSUE

MOLARITY OF LiCl	EFFECT ON RESPIRATION	EFFECT ON ANAEROBIC GLYCOLYSIS	EFFECT ON AEROBIC GLYCOLYSIS, %
0.01	o	o	+10
0.02	o	o	+30
0.05	o	o	+75
0.20	-10%	o	+100-125
0.40	-30%	o	+140
1.00	?	+10%	+200

fected. The injection dose was selected to correspond on a weight basis with the experiments of Good (5) on cats, namely 60 mg/kg. Eight animals were injected, 2 of which were injected with Li<sup>+</sup> as LiCl, 2 as LiCO<sub>3</sub>, 2 as LiSO<sub>4</sub> and 2 controls with NaCl. No acute symptoms were noted at this injection level and the dose was doubled (120 mg/kg.). The results are shown in table 2. Within 24 hours, rat 1 developed marked hyper-irritability and weakness of the hind legs. It died shortly thereafter. Rat 2 developed exactly the same symptoms within 24 hours but did not die. Several hours later, the animal showed generalized weakness with loss of hyper-irritability and spontaneous movement. It recovered uneventfully. However, when this animal was given a dose corresponding to 180 mg/kg., it became markedly hyperactive after 22 hours, and developed spontaneous generalized tre-

mors. Pronounced weakness of the hind legs developed in 26 hours and respiration ceased at 27 hours. *Rats 3 and 4* were discarded because the LiCO<sub>3</sub> was insoluble and produced skin ulcerations at the site of injection.

*Rats 5 and 6* showed exactly the same symptoms as *rats 1 and 2* and died at 28 hours. As might be expected the rats injected with NaCl showed no signs of abnormal behavior. Gross inspection of the organs of the experimental animals at autopsy showed no obvious injury, except that bright, red blood mixed with the feces was found in the bowel of *rat 1*. Similarly, microscopic examination of the organs of the experimental animals showed no obvious lesions. Admittedly, this is a small number of experimental animals, but since all 4 succumbed with the same symptoms and since we were more interested in the chronic effects of lithium on the

TABLE 2. EFFECT OF LITHIUM ON MALE RATS

RAT	ORIGINAL WEIGHT gm.	AUTOPSY WEIGHT gm.	DOSE OF Li/ DAY mg.	TOTAL DOSE BE- FORE AUTOPSY mg.	TOXIC SIGNS
1	275	265	1		Anesthetic death
2	280	300	1	34	None
3	270	280	1	34	None
4	250	270	3	102	None
5	280	300	3	102	None
6	265	270	3	102	None
7	215	230	5	170	32nd day present <sup>1</sup>
8	220	270	5	170	31st day present <sup>1</sup>
9	220	220	5	170	None
11	310	270	10	240	14th day present <sup>1</sup>
12	290	250	10	240	18th day present <sup>1</sup>
13	320	310	15	120	7th day present <sup>1</sup>
14	240		15	135	Died 11th day present <sup>1</sup>
15	350	340	15	105	Died 8th day

<sup>1</sup> The signs of toxicity were invariably increased reflex excitability followed by paralysis of the hind limbs and death. One animal was seen to die with labored respirations of diminishing rate. Several of the toxic animals developed profuse nasal discharges and harsh rasping respirations.

reproductive system, the acute study was concluded at this point and chronic experiments begun.

Table 2 shows the effect of daily injection of subacute doses of LiCl in male rats. The daily administration ranged from 1 to 15 mg. subcutaneously for periods ranging up to 34 days. Approximately 14 days after the first injection, the males were placed with mature females of known breeding capacity and the mating results noted.

Up to the 3-mg. daily dose level and a total injection level of 102 mg., no toxic symptoms were noted in the animals and each male conceived normal litters with 2 females. Furthermore, each animal gained weight during the experiment. At the 5-mg. daily dose level and a total amount injected of 170 mg., toxic symptoms appeared in 2 of the 3 animals tested on the 31st and 32nd days, respectively. The animal showing toxic symptoms on the 32nd day conceived 2 normal litters, while the other animal exhibiting toxicity failed to impregnate in 2 cases. The 3rd ani-

mal of this group did not show toxic symptoms but, on the other hand, failed to conceive litters in 2 females.

At the 10- and 15-mg. daily dose level, every animal developed typical toxic symptoms ranging from the 8th to the 18th day, and all lost weight. Two of the 3 animals on the 15-mg. dose level died on the 8th and 11th days respectively. The 8-day animal did not show any symptoms prior to death. The remaining animal of this group started to show symptoms on the 7th day, when a total of 105 mg. had been injected. Of the 5 animals in the 10- to 15-mg. dose level group, only 1 sired a normal litter, and this animal failed to do so with another female. But 1 animal (no. 14) died before exposure to the female and another (no. 15) died on the day of exposure to the female. The remaining 2 animals of this group (11 and 13) probably were too weak for copulation and their failure to reproduce can thus be accounted for. In confirmation of this, the testes of all the rats were sectioned and examined for observations of the germinal epithelium. In all cases, the latter appeared normal with an abundance of mature spermatozoa in the tubules. As in the acute experiments, microscopic examination of the other organs disclosed no obvious lesions.

#### DISCUSSION

From these experiments, it is obvious that the lithium ion has effects *in vitro* and *in vivo* which can be construed as highly toxic. At the present time, it is difficult to explain the widely different effects of the ion on the glycolysis of the spermatozoa and upon that of rat testicular tissue. First, it should be emphasized that lithium inhibits both the aerobic and anaerobic pathways of the degradation of glucose to lactic acid in the spermatozoa, whereas it affects only the aerobic glycolysis in testicular tissue and that in stimulatory fashion instead of inhibitory. It should be remembered, however, that a suspension of spermatozoa consists of homologous cells, whereas testicular tissue is composed of several cell types, the germinal epithelium and interstitial tissue being examples of widely different forms so far as morphology and function are concerned. Furthermore, we have already demonstrated (13) that it is difficult to dissociate the aerobic and anaerobic glycolysis of human spermatozoa. There is no evidence that the two pathways can be affected selectively and no Pasteur effect is apparent in these cells. But there are certain analogies between the carbohydrate metabolism of human spermatozoa and of muscle (13) which would indicate that the enzymatic components are probably similar if not the same. The effect of certain cations, particularly K<sup>+</sup>, has been demonstrated in brain (10). The aerobic glycolysis of that tissue is raised above the anaerobic glycolysis in the presence of excess K<sup>+</sup>, whereas the anaerobic glycolysis is inhibited. These workers did not use lithium and it is not likely that the effects of lithium we have found in testicular tissue and in the spermatozoa are analogous. In fact, we can state that the K<sup>+</sup> concentration in the environment of the spermatozoa can be increased at least one hundred fold without affecting the glycolysis or motility to any appreciable extent (14). Human spermatozoa are able to withstand relatively large changes in their osmotic environment, both on the hypotonic and hypertonic sides (14). It is therefore more suggestive of a specific ionic effect that

the lithium ion, in such low concentrations, can produce the effects described here on the spermatozoa.

In regard to the acute and chronic effects of lithium on normal rats, muscular weakness is one of the striking symptoms. This is true also in human toxicity (3). In fact, the symptoms produced in the rat are strikingly similar to those found in the human subject whose sodium chloride intake has been replaced by lithium chloride. It is significant, perhaps, that if one extrapolates, on a weight-for-weight basis, the chronic lithium dosage producing the symptoms in rats to the human being, the theoretical intake necessary to produce the human symptoms would be from 1.3 to 2.3 gm. daily. This corresponds rather closely with the intake in the human cases reported. Good (5) has shown in the cat that by far the greatest amount of Li<sup>+</sup> retained in that animal was to be found in the muscles. Our evidence in the rat points definitely to retention of Li, and the most pronounced symptoms produced are muscular in nature. If the analogy from the spermatozoa be projected, it would not be difficult to assume an effect on carbohydrate metabolism in muscle when a critical level of lithium is reached. This would be critical if cardiac muscle were affected in similar fashion, since lithium as a salt substitute is likely to be used as a salt substitute in many cases of cardiac insufficiency. It is distinctly possible, on the other hand, that lithium produces effects on the central nervous system which would produce certain of the effects found both in the rat and human subject.

#### SUMMARY

Lithium chloride, in low concentration, inhibits the aerobic and anaerobic glycolysis of human spermatozoa and destroys their motile activity. The lithium ion, in contrast, stimulates the aerobic lactic acid production of rat testicular tissue without affecting the anaerobic glycolysis or the respiration of that tissue. The acute and chronic effects of lithium chloride injected subcutaneously into male rats are described. It is shown that the symptoms induced in rats are strikingly similar to those found in lithium toxicity in the human being.

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# ACETALDEHYDE METABOLISM AND LIVER CHANGES IN DOGS MAINTAINED ON A PURIFIED DIET<sup>1</sup>

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**T**HREE are present in animal tissues at least four different enzyme systems capable of utilizing acetaldehyde as a substrate. These are: 1) one or more flavo-proteins similar to xanthine-aldehyde oxidase; 2) an aldehyde mutase requiring DPN as a hydrogen carrier; 3) animal carboxylase containing diphosphothiamine; and 4) aldolase. All of these could theoretically be involved in the metabolism of acetaldehyde, but previous studies (1) have indicated that the carboxylase system does not play a major role in this process. The mutase and oxidase are concentrated in the liver, and the liver is known to be a major site of acetaldehyde metabolism (1). Since both of these enzyme systems are dependent upon dietary factors, it seemed possible that a study of acetaldehyde metabolism during niacin or riboflavin deficiency would aid in assessing the relative importance of each of these enzymes in the metabolism of acetaldehyde.

The experiments herein reported resulted from such an attempted investigation, but it soon became evident that the abnormal acetaldehyde metabolism which resulted from the feeding of a purified diet was unrelated to niacin or riboflavin deficiency. When dogs were fed a purified diet containing adequate niacin and riboflavin, the rate of disappearance of administered acetaldehyde was slowed. A degenerative change in the liver, characterized by a loss of cytoplasmic staining properties and by a swelling of the liver cords with obliteration of the sinuses, was found in those dogs showing an abnormal acetaldehyde disappearance curve. Other liver function tests were normal.

## EXPERIMENTAL

The basic diet for this study is shown in table 1. In some of the experiments the tocopherols were omitted without apparent effect. The nicotinic acid-deficient diet was prepared by omitting the nicotinic acid. The low protein diet contained 8 per cent casein and 39 per cent sucrose. The high protein diet contained 35 per cent casein and 24 per cent glucose. In one series of experiments an attempt was made to inhibit intestinal bacterial synthesis of growth factors by injecting the B vitamins subcutaneously rather than incorporating them in the diet. In this latter series the foodstuffs and choline were fed orally while the subcutaneous vitamin mix-

Received for publication January 4, 1949.

<sup>1</sup>This work was supported by grants from the Hendricks Research Fund and from the Nutrition Foundation, Inc.

ture<sup>2</sup> administered weekly provided 0.5 mg. each of thiamine, riboflavin and pyridoxine, 2.5 mg. of calcium pantothenate, and 12.5 mg. nicotinamide per kg. body weight per week.

Before young adult dogs were placed on one of these diets and at arbitrary intervals during the dietary regime, the acetaldehyde disappearance curve was determined. This 'load test' was run as follows. A 4 per cent solution of acetaldehyde was prepared by diluting 51.3 cc of freshly distilled acetaldehyde to one liter with cold 0.8 per cent NaCl. The dog was lightly anesthetized with 0.8 cc/kg. of 3.25 per cent nembutal intravenously because of the irritation accompanying the acetaldehyde administration. Previous studies have shown no effect of anesthesia on the rate of acetaldehyde metabolism. Two cc/kg.<sup>3</sup> of the 4 per cent acetaldehyde were injected intravenously over exactly 3 minutes, and blood samples were removed from the jugular vein at exactly 3, 5, 8 and sometimes 12 minutes after completing the injection. A 1:10 tungstic acid filtrate was rapidly prepared from each oxalated blood sample, and the protein precipitate was removed by centrifuging in a stoppered tube in the cold room. Loss of acetaldehyde from the samples by volatilization was minimized by keeping all tubes stoppered and in an ice bath at all times possible.

TABLE I. COMPOSITION OF SYNTHETIC BASAL DIET FOR ACETALDEHYDE STUDIES

Casein <sup>1</sup> .....	19 gm.%	Nicotinic acid.....	2.5
Cod liver oil.....	2	Calcium pantothenate.....	0.5
Cottonseed oil (Wesson).....	7	Riboflavin.....	0.4
Salt mix (Phillips and Hart).....	4	Thiamine chloride.....	0.2
Sucrose.....	28	Pyridoxine hydrochloride.....	0.2
Glucose.....	40		
Choline chloride.....	100 mg.%		
34% mixed tocopherols <sup>4</sup> .....	10		

<sup>1</sup> Vitamin Test, General Biochemicals, Inc.

<sup>2</sup> Distillation Products, Inc.

The acetaldehyde in the filtrate was distilled and determined by the method of Stotz (2).

Liver total lipid was determined by weighing the chloroform-soluble extractives from dried liver. Bromsulfalein excretion (3), prothrombin time (4), and serum alkaline phosphatase (5) were determined as indicated. Biopsy or fresh autopsy sections of the liver were fixed in 10 per cent formalin for histological study.

#### RESULTS

Figure 1 shows some typical acetaldehyde disappearance curves. The two lower lines represent the range in 'normal' dogs (up to 6 mg.% at the 3-minute point), while the zone between curves II and III is considered a moderate elevation; blood levels above III are clearly abnormal, while those in the region of V and VI are often associated with a fatal termination. For convenience in subsequent discussions the blood acetaldehyde level at the 3-minute point will be used to indicate the location of the acetaldehyde disappearance curve on figure 1.

<sup>2</sup> Solu-B with added pyridoxine, Upjohn Company.

<sup>3</sup> Early doses of 3 cc/kg. of 4% acetaldehyde produced a high percentage of fatalities among the deficient dogs.

Most dogs tested at weekly, bi-weekly, or longer intervals continued to show normal acetaldehyde disappearance curves until the first appearance of a delayed metabolism, after which high curves were consistently obtained. Moderately elevated blood acetaldehyde levels were not considered significant because of occasional spontaneous fluctuations between the normal and moderately elevated zones. Very rarely a dog would show a single high curve, and then spontaneously drop back into the normal range. Considerable spontaneous variation was noted from week to week in the exact level of the abnormal curves; no particular significance could be attached to this type of fluctuation within the abnormal zone.

Of 46 mongrel adult dogs chosen at random and studied in this investigation, the initial acetaldehyde disappearance curve was normal in 35, moderately elevated (7-11 mg.%) in 8, and definitely high (16-25 mg.%) in 3 dogs. Twenty-seven of the normal dogs were maintained on one of the purified diets for at least 8 weeks; 15 of these eventually developed high curves (16-25 mg.%), 3 were moderately elevated (9-11 mg.%), and the remaining 9 dogs continued to show normal acetal-

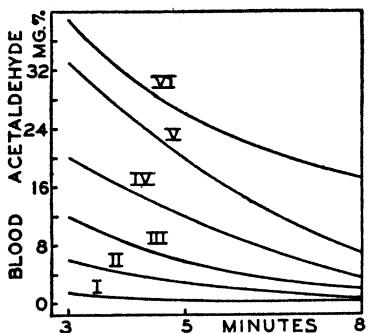


Fig. 1. TYPICAL ACETALDEHYDE DISAPPEARANCE CURVES IN DOGS. Two cc/kg. of 4% acetaldehyde were injected i.v. over 3 minutes; blood samples were analyzed for acetaldehyde 3, 5, and 8 minutes after completing the injection. Disappearance curves falling in the zone between curves I and II are normal; above curve III is clearly abnormal.

dehyde disappearance curves throughout the dietary regime. Four out of 5 dogs starting with moderately elevated levels developed high curves (14-31 mg.%) when maintained on a purified diet. One of the dogs starting with a high curve was given a variety of dietary factors over a period of 6 months without permanently lowering it, and this dog eventually died following a very high blood acetaldehyde (38 mg.%).

Ten deaths in this series were attributed to the acetaldehyde administration, and all of the deaths followed high acetaldehyde curves (usually 30-40 mg.%). Three deaths resulted from respiratory failure while the experiments were being run, but this cause of death was eliminated in the others by routinely giving artificial respiration to all dogs showing signs of a high curve (slow heart rate and respiratory failure). The latter deaths occurred after the blood acetaldehyde had fallen to reasonably low or negligible levels. Some of the dogs died following the first or second high acetaldehyde curve, while others survived repeated high blood acetaldehyde levels.

The basal diet, the nicotinic acid-deficient diet and the subcutaneous vitamin regime all gave comparable results in producing an abnormal acetaldehyde metabolism. Five dogs maintained on the basal diet developed high curves within approx-

imately 12 weeks; 6 other dogs were maintained on the same diet for approximately 20 weeks without developing this abnormality. Maintenance on a nicotinic acid-deficient diet (supplying single nicotinic acid injections as the deficiency became severe in order to maintain life) resulted in high curves in 4 dogs within 4 to 9 weeks, while 4 other dogs continued to show a normal acetaldehyde disappearance after 9 to 17 weeks. Of 5 dogs that were given both the basal and nicotinic acid-deficient rations at different times during their dietary history, 2 developed high curves in 17 to 19 weeks; 2 remained normal for at least 26 weeks, and 1 dog was continued for 100 weeks without showing abnormal acetaldehyde metabolism. Feeding the basal diet to which 1 per cent sulfasuxidine had been added produced a high curve in one dog in 11 weeks, but not in another dog that was continued for 19 weeks. Of 6 dogs that were fed the purified foodstuffs orally and given the vitamin supplement subcutaneously, 4 developed high curves within 10 to 22 weeks, while the other 2 remained relatively normal for 26 weeks.

Five of the dogs maintained on the basal or subcutaneous vitamin diet for 20 to 26 weeks without developing an abnormal acetaldehyde metabolism were then placed on the low protein diet. Two of the dogs developed high curves in 3 and 11 weeks respectively; the other three still had normal disappearance rates after 11 to 17 weeks on low protein. A sixth dog that did not develop a high acetaldehyde curve during 100 weeks on the basal diet did so after three weeks on the low protein diet. These results indicate that a low protein diet aids in establishing the abnormal acetaldehyde metabolism.

Practically all of the dogs on the basal or subcutaneous vitamin diet increased in weight. Changes in body weight on the low protein diet were small. Body weights fluctuated widely on the nicotinic acid-deficient diet as nicotinic acid was withheld or administered. The appearance of abnormal acetaldehyde curves could not be correlated with such weight changes. Both normal and abnormal disappearance rates were observed at all phases of the growth response in different dogs.

*Liver Studies.* A number of dogs showing normal acetaldehyde disappearance curves (2-5 mg. %) were tested for bromsulfalein excretion, plasma prothrombin time and serum alkaline phosphatase just before they were put on the basal ration or the subcutaneous vitamin regime. After subsisting on the purified diet for approximately 14 weeks, the acetaldehyde test was repeated, and from the group were chosen 5 dogs showing definitely high acetaldehyde curves (20-25 mg. %) and 3 dogs still showing normal curves (4-6 mg. %). The above liver-function tests were repeated in these dogs, and a liver biopsy was then removed from each under aseptic conditions.

Bromsulfalein retention in the group of dogs at the start of the experiment averaged 1.55 mg. %, and did not change significantly during the dietary period. The prothrombin time of  $12\frac{1}{2}$  per cent plasma was essentially the same (22 sec.) in both the normal and high acetaldehyde group. Serum alkaline phosphatase averaged 1.15 units in the group before starting the experiment; the normal group did not change during the dietary period, but each of the dogs that developed a high curve also showed a perceptible increase in serum phosphatase, the abnormal group averaging 2.48 units at the time of biopsy. Liver lipid and water content were normal (5.7%

lipid, 31% total solids) in a dog running a high acetaldehyde curve (28 mg. %), and there was no gross or histological evidence of fatty livers in any of the adult dogs maintained on these diets.

*Histological Studies.* The livers were studied histologically without reference to biochemical changes. After fixation in 10 per cent formalin, paraffin sections were made and stained simultaneously with hematoxylin and eosin. The sections were grouped into three categories: normal, definitely abnormal and intermediate. The principal features studied were cell size, liver cord arrangement, sinusoid size and cytoplasmic staining qualities. All of these were altered in the abnormal and intermediate groups, the difference being one of degree.

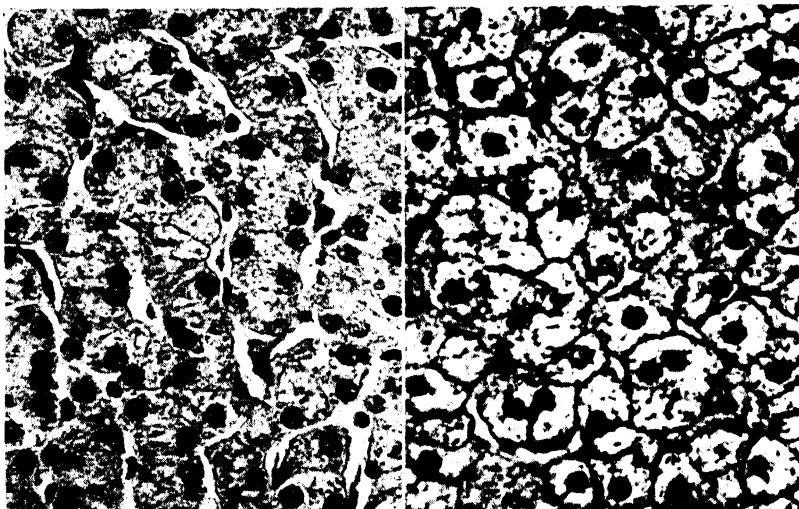


Fig. 2 (left). NORMAL DOG LIVER.  $\times 525$ .

Fig. 3 (right). LIVER FROM DOG maintained on synthetic basal diet.  $\times 525$ . Section shows severe hydropic degeneration.

The normal dog liver (figure 2) has fairly straight liver cords radiating from the central vein, regular sinusoids and uniform cells with distinct boundaries and fine eosinophilic granules in the cytoplasm. The livers which were classified as abnormal (figure 3) appeared disorganized. Liver cords were indistinguishable and sinusoids were not apparent. The outline of the individual cells was very distinct because of a characteristically deeply stained material which appeared to be the cell membrane. Because they were considerably larger than normal they encroached upon the sinusoids and either distorted or obliterated them. The cytoplasm was rarified except for a few scattered threads and granules of eosin stained material. There were no distinct vacuoles. Stains for fat (Herxheimer Scarlet Red) and glycogen (Best's Carmine) did not show any appreciable abnormalities.

Many of the dogs maintained on the basal ration for several months showed some degree of this so-called hydropic degeneration of the liver, but the livers of all

dogs were not invariably altered pathologically. Occasional dogs have been maintained on the diet for long periods of time without developing abnormal changes in the liver. There was a general tendency for abnormal acetaldehyde disappearance curves to be associated with these degenerative changes in the liver. Of 6 dogs that continued to show normal acetaldehyde metabolism while being maintained on the diet for 3 months or longer, 3 had normal livers and 3 showed moderate degeneration. Of 7 dogs that gave elevated acetaldehyde curves at the time of liver biopsy, 4 showed moderate degenerative changes in the liver and 3 had severe hydropic degeneration. The appearance of high acetaldehyde curves and liver pathology bore no relationship to the amount of acetaldehyde previously administered.

*Effect of Dietary Supplements.* Dietary supplements were evaluated in terms of the effect obtained on the elevated acetaldehyde disappearance curve. Following the first appearance of a high acetaldehyde curve, additional tests were made at weekly intervals to establish the consistent nature of the abnormal change. The dietary supplement was then given orally or subcutaneously for periods varying from one to 12 weeks, and the acetaldehyde test was repeated at weekly or bi-weekly intervals during the period of supplementation. When the supplement was ineffective in restoring the curve to normal, it was replaced by another test substance. A positive response to the supplement was considered to be significant when the initial high curves, exceeding 12 mg. per cent for several weeks prior to testing, were reduced to normal (6 mg. % or less) and maintained there during the period of supplementation for at least 3 to 4 weeks. According to this criterion, a high casein diet cured 3 of 5 dogs tested. Inositol gave some positive, but erratic effects without permanent cures. Other substances tested were without effect.

Table 2 summarizes the results obtained in some of the tests with dietary supplements, particularly protein and inositol. Methionine was also tested and found negative in 2 dogs that were later found to be refractory to the high protein diet. The high protein diet had to be fed for 5 to 6 weeks to some dogs in order to obtain an effect. The results are clear in assigning a beneficial role to large amounts of casein in this condition.

The beneficial response to inositol was erratic, unpredictable and temporary. Reasonably normal curves first produced by inositol administration were not sustained in spite of continued inositol administration. In some dogs inositol had no beneficial effect at all; in others it was beneficial the first time it was administered but subsequent high curves in the same dog were unaffected by repeated administration of inositol either orally or subcutaneously.

Negative tests were obtained with biotin, p-aminobenzoic, folic acid, tocopherols, menadione, ascorbic acid, cystine, ergostanyl acetate, a 4-fold increase in choline, and a mixture of trace elements. The results with protein and inositol demonstrate that the condition is sometimes reversible by dietary means, but some dogs have been refractory to natural supplements such as Wilson's liver L, fresh liver, Brewer's yeast, milk and horse meat. For example, 2 dogs maintained their body weights on a diet of raw milk exclusively for 6 weeks, but continued to show high acetaldehyde curves throughout this period. This failure to respond to natural supplements may represent an irreversible stage of liver damage. Some of the negative

tests are equivocal because there is no assurance that any dietary factor would have yielded a cure at the time of the test.

*Alcohol and Acetoin.* Two dogs averaging 20.2 kg. were maintained on the nicotinic acid-deficient diet for 8 weeks. At this point they averaged 16.0 kg. and both showed high acetaldehyde curves. The average rate of alcohol metabolism was determined (6) to be 10.6 mg. % per hour following an oral dose of 15 cc. of 19 per cent (by volume) alcohol per kg. After continuing on the same diet with a large excess of nicotinic acid for two weeks, body weights increased to 18.2 kg., and the

TABLE 2. EFFECT OF DIETARY SUPPLEMENTS IN RESTORING THE ACETALDEHYDE DISAPPEARANCE CURVE TO NORMAL

WEEKS OF DIET AND TYPE <sup>1</sup>	AcH CURVES BEFORE ADMIN. OF TEST SUBSTANCE <sup>2</sup>	SUBSTANCE TESTED		AcH CURVES DURING ADMIN. OF TEST SUBSTANCE <sup>2</sup>
		Name	Mg./Kg. Body Wt. Daily	
9 N	* 16-28	Inositol	22	8-6
		PABA	7	
		Biotin	0.16	
100 B + 5L	23-29-31	High prot.	Ad. lib.	**24**4-5-5
4 N	* *-27	Biotin	0.17	41 (fatal)
15 S	17-11-20	Inositol	56	6-6-10-13
20 B + 5L	13-17-15	High prot.	Ad. lib.	**8-5-5-6-4
17 B	* 22-13	Inositol	81	*7-21-10**22
		High prot.	Ad. lib.	*31**6-7-5-4
16 B	* 25-21	Inositol	80	**28
		Methionine	160	27-8*18*30
		High prot.	Ad. lib.	**31**11*10
15 B	* 25-17	Inositol	66	*9-12-27**18
24 V	24-14-15	Tocopherols	47	11-15-18
		Vit. K	1.2	
24 V	18-16-15	P. A. factor	½ unit	11-10-10
14 V	* 20-26	Inositol	83	*35-35
		Methionine	166	37-37*31*26
		High prot.	Ad. lib.	**35**29*14
13 B	* 20-20	Inositol	63	*37-9-21**13

<sup>1</sup> Diet abbreviations: B = basal; N = nicotinic acid deficient; L = low protein; S = sulfasuxidine; V = vitamins subcutaneously.

<sup>2</sup> Each figure refers to mg. % blood acetaldehyde at 3-minute point, determined at weekly intervals. \* indicates a week in which no curve was run.

alcohol disappearance rate increased to 15.5 mg. per cent per hour; the acetaldehyde disappearance curves remained high. The change in the rate of alcohol metabolism following treatment with nicotinic acid was probably significant, but it should be noted that the rate observed during the deficiency was not exceptionally low as compared with the average expected of a group of 'normal' dogs. Blood acetoin following the administration of 3 cc. of 4 per cent acetaldehyde per kg. was determined (7) in several nicotinic acid-deficient dogs showing delayed acetaldehyde disappearance curves. As expected from earlier results (1), the high blood acetaldehyde (35 mg. %) was accompanied by high levels of blood acetoin (0.5-0.7 mg. %).

*Other Species.* Acetaldehyde metabolism was studied in rats by injecting intraperitoneally 0.5 cc. of 4 per cent acetaldehyde per 100-gm. body weight. Blood samples obtained by decapitation were analyzed, and the averages of the individual results obtained were 18, 9 and 2.5 mg. % acetaldehyde respectively at the 3-, 5-, and 10-minute points. This same normal curve was obtained in rats fed Purina dog chow for 41 and 75 days after weaning, and in a parallel group of rats fed the purified nicotinic acid deficient ration. Other weanling male rats were placed on the purified basal diet previously described and killed at monthly intervals. Histological sections of the livers stained with eosin and hematoxylin were normal throughout the 4-month dietary regime; liver lipids were similarly normal, averaging 15.6 per cent of the total solids.

Two monkeys that were being killed after several years of vicarious existence in the laboratory were subjected to the same acetaldehyde load test used in the dogs. In comparison with the curves obtained in dogs (fig. 1), one monkey had a slightly elevated curve (8 mg. %) while the other was markedly elevated (24 mg. %).

#### DISCUSSION

Some, but not all, adult dogs maintained on a typical purified diet develop a defect in acetaldehyde metabolism that is reflected in a delayed disappearance curve. The nature of the dietary deficiency responsible for this effect has not been established clearly. A low casein diet aids in establishing the pathology, and a high casein diet restores a certain percentage of animals to normal in this respect. Nevertheless, it is difficult to believe that this syndrome is a manifestation of protein deficiency per se. It can be produced in adult dogs on a 19 per cent casein diet (supplying approximately 18% of the calories) and none of the dogs show protein deficiency changes in body weight or plasma proteins. The response to the high casein diet is slow. The casein could be a carrier for some unknown nutritional factor, or the protein content of the diet could play an accessory rôle in the production of some other deficiency.

The altered histology of the liver is called hydropic degeneration despite the small or negligible changes in water content. Such degenerative changes in the liver were previously described in dogs fed a low protein diet (8, 9). The present study indicates that protein deficiency is not the only factor capable of producing hydropic degeneration, since it was obtained with diets containing 19 per cent casein. The altered acetaldehyde metabolism could have been due to changes in the gross architecture of the liver or to a specific biochemical lesion in one of the enzyme systems concerned with acetaldehyde metabolism.

#### SUMMARY

Fifty to 60 per cent of the adult dogs maintained on a purified diet containing the usual B-complex vitamins developed a defect in acetaldehyde metabolism that could be demonstrated by an acetaldehyde load test. A low casein diet helped to produce this deficiency but it could be obtained on a 19 per cent casein intake. The delayed acetaldehyde disappearance curve was not associated with body-weight changes but was correlated to some extent with the appearance of hydropic degenera-

tion in the liver. Other evidence of liver damage was not prominent; liver fat, bromsulfalein excretion and prothrombin times were normal; serum alkaline phosphatase may have been slightly elevated. Once this defect was produced, the acetaldehyde disappearance curve could be restored to normal in some dogs by the prolonged administration of a 35 per cent casein diet; it was also benefited sporadically by inositol. Disappearance curves in other dogs could not be restored to normal even by feeding natural foodstuffs for moderate periods of time.

Weanling rats grown on the same ration did not show any abnormalities in acetaldehyde metabolism or liver histology.

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# EFFECT OF CALORIC RESTRICTION ON THE ADRENAL RESPONSE OF OVARIECTOMIZED C<sub>3</sub>H MICE

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**W**OOLLEY, Fekete and Little (1) reported that certain strains of mice develop nodular hyperplasia of the adrenal cortex following ovariectomy. Kirschbaum, Frantz and Williams (2) found that such changes occur spontaneously in the NH strain in relation to the early ovarian failure seen in this strain. The change can be induced even earlier by ovariectomy. These facts suggest that the adrenal may be responding to pituitary stimulation caused by low estrogen levels in the body fluid. As the adrenal changes develop the uterus and vagina show increasing evidence of estrogen stimulation. The adrenal is, presumably, the source of the estrogen.

These facts have a bearing on caloric restriction since a number of investigators have interpreted their findings as indicating that dietary restriction inhibits the pituitary causing various degrees of gonadal failure; see Moore and Samuels (3); Werner (4); Mulinos and Pomerantz (5); Huseby, Ball and Visscher (6); and others cited in the above papers.

In this study an attempt was made to determine whether the partial or total anterior pituitary inhibition which is believed to prevail in caloric restriction is of such a nature as to prevent the adrenal changes known to follow ovariectomy in certain strains of mice. It was also important to determine whether restriction influenced estrogen production in case the adrenal changes developed in mice on restriction.

Since the effects of pure caloric restriction on the C<sub>3</sub>H mouse have been extensively investigated in this laboratory (6, 7) and since this strain shows the typical adrenal response following ovariectomy it was used exclusively in this study.

## MATERIALS AND METHODS

C<sub>3</sub>H mice were ovariectomized at 21 to 23 days of age. They were housed individually in an air-conditioned room. The diet used was that described by Visscher *et al.* in their study on the incidence of mammary carcinoma. The controls had unlimited access to food. The restricted mice were fed a pellet formed in a brass mold calibrated to measure the required amount for one day. One group was restricted to approximately 66 per cent of the calories eaten by the controls; another group was restricted to approximately 50 per cent. Tap water was continually available to both restricted and control groups. Each group contained at least 50 mice.

Received for publication February 3, 1949.

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<sup>2</sup> Aided by a grant from the U. S. Public Health Service.

It should be noted that the restricted mice received approximately the same amount of protein, vitamins and salts as the controls. Animals were weighed each week. Vaginal smears were made by the lavage method. Sections were made of the uterus, vagina and adrenals.

*General observations.* Restricted mice were uniformly more active than full-fed mice. They were also more irritable when handled. The restricted ration was promptly eaten by a mouse in good health. There were some perianal infections in the full-fed groups which yielded to prompt washing with boric acid solution and painting with tincture of metaphen. There was no evidence of poor health in restricted animals as compared to controls. Fur, skin, eyes, nose and mouth showed no abnormality.

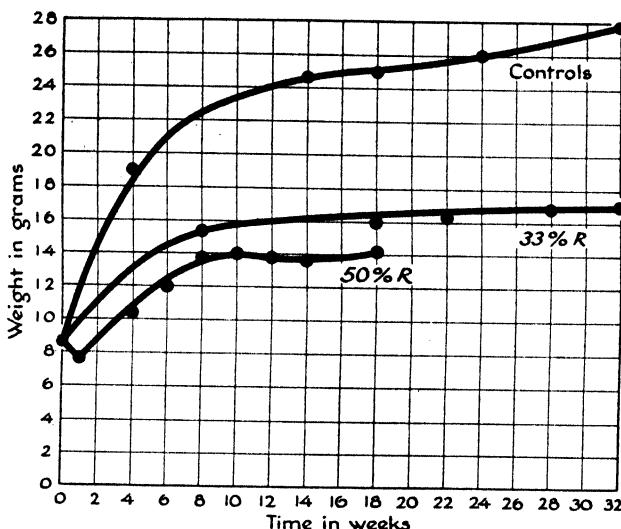


Fig. 1. GRAPH OF BODY WEIGHT of mice fed *ad libitum*, at 33% and 50% caloric restriction.

*Growth rate.* In figure 1 the rate of growth of restricted and control group is shown up to the 32nd week. The rapid growth phase persists longer in the controls. In the group restricted to 50 per cent there is a loss in the first week which is recovered in the second. Thereafter the curve for this group resembles that of the group under less severe restriction but is at a lower level.

*Vaginal smears.* The restricted animals never developed a positive smear. At 37 days after ovariectomy the controls began to show evidence of stimulation; in 4 months they showed a dense, mixed smear composed of leucocytes, epithelial and cornified cells. Beyond this time there is no essential change in the character of the smear. The cellular content of the smear is non-cyclic in contrast to normal estrus cycles seen in the intact, full-fed animal.

*Adrenals.* By 37 days after ovariectomy histological changes could be observed in both restricted and control groups. The changes were further advanced in the controls. There was proliferation of small, dark subcapsular cells which invade the

cortex. When this type of cell surrounds large, clear cells the result is that described by Woolley and Little (8) and noted by Kirschbaum, Frantz and Williams (2) in the NH strain. At the 3-month stage the changes were further developed in both restricted and controls. At this time there was no qualitative difference between the two groups although there was a larger fraction of the cortex involved in the controls. The X zone disappears very early in that part of the gland showing the above histological changes. Grossly the glands showed white or yellowish areas, sometimes bulging on the surface.

*Uterus.* By the time the vaginal smears showed the dense, mixed picture the uterus of the control mouse was grossly enlarged and hyperemic while that of the restricted animal was completely atrophic. Sections of the control organ showed evidences of estrogenic stimulation, i.e., tall columnar epithelium, infolding of the luminal surface, numerous well developed glands, loose stroma, etc. In the restricted mouse the histologic picture is one of hypoplasia.

*Vagina.* In the restricted mouse the vagina remained very small with usually not more than two rows of epithelial cells, except deep in the folds. The full-fed animal showed a well-developed vagina at 99 days with an epithelium many layers deep. Mucification was prominent in some animals with some cornification. Later stages showed more pronounced cornification.

*Refeeding.* Five of the ovariectomized restricted mice were fed *ad libitum* after 12 weeks of restriction at which time their weight varied from 17.7 gm. to 20.0 gm. They came into estrus after 29 to 35 days of full feeding at body weights between 22.2 gm. and 26.4 gm.

#### DISCUSSION

This experiment was based on the assumption that the controls would eat on the average 2.66 gm. per day after the second week. They actually ate slightly more than the expected amount. The restricted animals therefore were subjected to a caloric restriction very slightly in excess of that planned.

The outstanding difference between the full-fed and restricted ovariectomized mouse is that the former develops evidence of estrogenic stimulation while in the latter the uterus and vagina remain atrophic. Both develop qualitatively identical histological changes in the adrenals. Since our unpublished experiments show that the restricted mouse is as sensitive to estrogens, in vaginal and uterine response, as the full-fed animal one is forced to believe that it does not produce enough estrogen to cause stimulation.

The concept of pseudohypophysectomy advanced by earlier workers seems adequate to explain the failure of ovarian function seen in the restricted, intact mouse since the response to gonadotropin or pituitary implants is prompt. Stated in its simplest form it means that restriction inhibits at least that function of the pituitary which controls the ovary.

No such simple explanation is adequate, however, when applied to the facts observed in the tumor-bearing, restricted castrate. This animal has adrenal cortical tumors qualitatively identical with those seen in the full-fed mouse but there are no signs of estrogen production. It is true that controls usually show more tumor

tissue in the cortex than do restricted mice. However, we have seen control animals with the usual evidences of estrogenic stimulation but having less tumor tissue than seen in some restricted mice failing to show such evidences. Hence it is improbable that the lesser amount of tumor tissue seen in the restricted animal explains failure to produce estrogens.

In the present state of knowledge caution must be used in interpreting these facts. It seems very probable that the tumors are the site of estrogen production in full-fed castrates of certain strains and in the NH mouse where they occur spontaneously. However, the fact that the tumors occur in the restricted castrate without evidence of hormone production makes it clear that the factors which are responsible for the histological change and for the secretory activity are not identical. Whether suppressed pituitary activity in the case of the restricted mice is the determining difference cannot be answered finally on the basis of these experiments. Our efforts to bring about estrus in the restricted tumor-bearing mouse with gonadotropin (9) and with adrenocorticotropin (10) were not successful. Likewise we were not able to produce estrus with fresh pituitary implants (unpublished data). The results of Boutwell, Brush and Rusch (11) indicate that restriction may inhibit one pituitary function while enhancing another.

#### SUMMARY

The ovariectomized C<sub>3</sub>H mouse develops adrenal cortical adenomas whether full-fed or restricted. The restricted tumor-bearing mouse fails to show stimulation of the uterus and vagina while the full-fed animal shows constant stimulation following the development of histological changes in the adrenal cortex. Where the caloric block to estrogen production takes place and what rôle the pituitary plays remain unsolved problems.

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# SECRETION OF INSULIN AND OF A HYPERGLYCEMIC SUBSTANCE STUDIED BY MEANS OF PANCREATIC-FEMORAL CROSS-CIRCULATION EXPERIMENTS<sup>1</sup>

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**T**HE hypoglycemic phase of the normal glucose tolerance curve is generally attributed to excessive compensatory reduction of the blood sugar level, brought into play by the hyperglycemic phase. Investigators, however, do not agree as to whether this is accomplished by stimulation of insulin production (1-5) or through a protracted inhibition of liver glycogenolysis (6). The solution of this problem is important for academic and practical reasons, for if one believes that hyperglycemia stimulates the insular tissue and that a continuous overactivity may result in exhaustion, one must control both diabetes mellitus (7-8) and hyperinsulinism (9) with 'painsstaking care' (7). On the other hand, if one believes that changes in the rate of liver glycogenolysis is the paramount factor, the strict control of the blood sugar level and of glycosuria may become less important.

## METHODS

The problem was investigated by means of pancreatic-femoral anastomoses. Seventy dogs were used. All animals were fasted for 16 hours preceding the experiment and were anesthetized with sodium amytal (70 mg/kg. intramuscularly, plus a single 50 to 100-mg. dose intravenously, if needed). This barbiturate was selected because it is reported to have little or no effect on blood sugar concentration (10, 11). The experiments were performed as follows. A donor *dog A* was prepared by cannulating the pancreaticoduodenal and one of the femoral veins. The other femoral vein was exposed for withdrawal of blood samples. A recipient *dog B*, smaller than *dog A*, was prepared by cannulating the femoral vein and artery on one side and exposing the femoral vein on the other. The blood pressure of the dogs was measured with mercury manometers connected with the carotid arteries. Having thus prepared the animals, a control sample of blood was taken, the animals were heparinized,<sup>2</sup> the pancreaticoduodenal vein of *dog A* was connected with the femoral vein of *dog B* and the femoral artery of *dog B* with the femoral vein of *dog A*. The anastomoses were made with transparent polyethylene tubing and the free flow of blood ascertained by injecting a minute bubble of air into the tubing before every blood sampling. The pancreatic-femoral anastomosis was kept open continuously. The return flow from the artery of *B* to the vein of *A* was opened as needed to maintain arterial blood pressures of both dogs approximately equal to their initial values. Four samples of blood were taken at 15-minute intervals and, after this control period, 5 cc. of a 20-per cent solution (1 gm.) of glucose per kilo body weight or the same volume of iso-osmotic (4%) saline were rapidly injected into the exposed femoral vein of *dog A*. Blood samples were taken at 15-minute intervals for 75 minutes following the injection, the anastomoses were then disconnected and the sampling continued 15 minutes later and then every 30 minutes for 2 more hours. Blood sugar was determined in duplicate according to the method of Folin and Malmrose (12). In some control experiments a branch of the mesenteric vein of *dog A*, about equal in size to its

Received for publication October 21, 1948.

<sup>1</sup> A summary of this paper was presented before the meeting of the American Physiological Society, Minneapolis, Sept. 16 and 17, 1948.

<sup>2</sup> Heparin was generously supplied by the Wilson Laboratories of Chicago.

pancreaticoduodenal vein, was used instead of the latter. To minimize any difference in the rate of blood flow, cannulas of similar size were used in all experiments.

In other experiments alloxan diabetic dogs were used. The animals had received 70 mg. of alloxan per kilo of body weight by intravenous injection at least 3 days before the experiment and had had glycosuria for at least 2 days.

The statistical significance of all pertinent data was computed and expressed as 'Probability' (P).

#### RESULTS

The results are presented in the form of average blood sugar curves. Figure 1 represents the glucose tolerance curve of intact anesthetized and heparinized dogs.

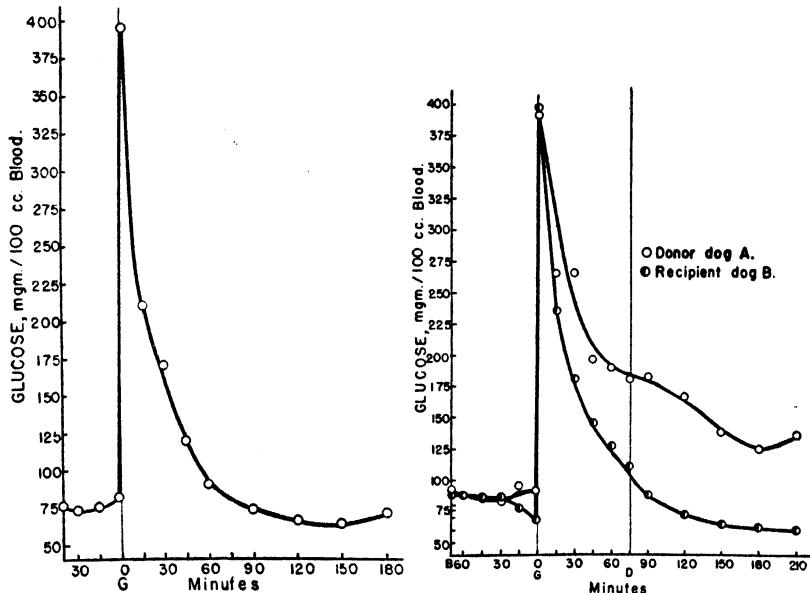


Fig. 1 (left). GLUCOSE TOLERANCE CURVE. Normal dogs. Average of 4 experiments. G: intravenous injection of 20% glucose solution (1 gm/kg. body weight).

Fig. 2 (right). PANCREATIC-FEMORAL ANASTOMOSIS. Normal dogs. Average of 3 experiments. B: before opening the anastomosis; G: intravenous injection of 20% glucose (1 gm/kg. body weight) into both dogs; D: anastomosis disconnected.

It demonstrates that, under the experimental conditions used, the carbohydrate metabolism of the animals was not altered significantly, as a normal curve could be obtained.

Figure 2 shows that when *dogs A* and *B* are connected by means of the pancreatic-femoral anastomosis and glucose is injected into both dogs simultaneously, the tolerance curve of *dog A* resembles a diabetic curve, whereas that of *dog B* is normal. The difference in the behavior of the two dogs shows that at least part of the insulin produced by the pancreas of *dog A* can be transferred to *dog B* and that the preparation used is suitable for this type of study.

Figure 3 shows the results of experiments in which *dogs A* and *B* were connected by a pancreatic-femoral anastomosis and glucose was injected only into the donor

*dog A.* The following phenomena will be observed: *a)* During the control period preceding the injection, the blood sugar of both dogs remained practically constant; *b)* following the injection of glucose into *A* the blood sugar of *B* started to decline sharply within 30 minutes, reaching a minimum value in from 60 to 90 minutes. This sharp decline was observed in all experiments and was even more marked than it would appear in the average curve. The latter is somewhat flattened because the beginning of the decline and the lowest blood sugar concentration did not happen at exactly the same time in all experiments.<sup>8</sup> The blood sugar of *B* remained low for about 2 hours after the two dogs had been disconnected, then gradually returned toward normal levels.

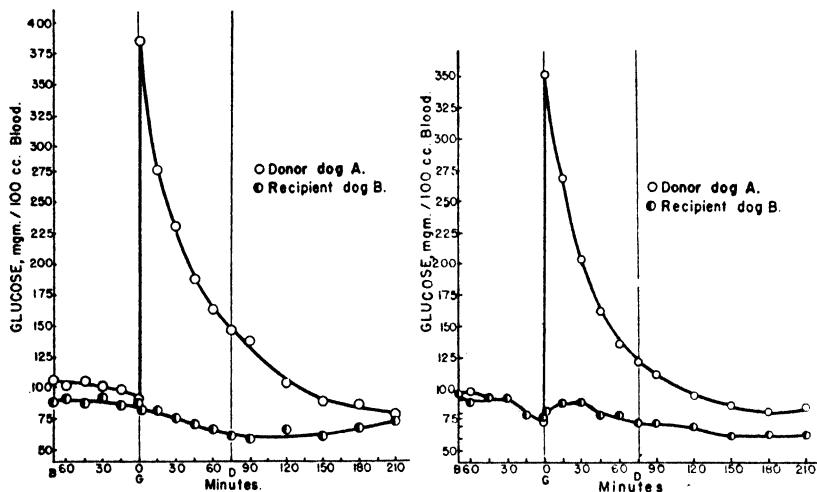


Fig. 3 (left). PANCREATIC-FEMORAL ANASTOMOSIS. Normal dogs. Average of 5 experiments. G: intravenous injection of 20% glucose (1 gm./kg. body weight) into the donor dog.

Fig. 4 (right). MESENTERIC-FEMORAL ANASTOMOSIS. Normal dogs. Average of 6 experiments.

It will be further noted that the decline in the blood sugar concentration of *dog B* followed the rise in blood sugar concentration of *dog A*, at a time when the glucose present in the pancreatic blood of *A* flowing into *B* tended to raise the glycemia of the latter rather than lower it. The hypoglycemia observed in *dog B* thus acquired even greater meaning. The fact that the decline in the blood sugar of *B* did not start until about 15 to 30 minutes after the blood glucose of *A* had been raised by the injection, that it reached minimum values in from 30 to 60 minutes and that it lasted for more than 2 hours after the animals had been disconnected suggests that the phenomenon is due to overproduction of insulin by the pancreas of *dog A* following the injection of glucose. The following experiments were performed to confirm this tentative interpretation.

It was necessary to rule out the possibility that the described phenomena may have been due to non-specific actions of the injection per se on the pancreas of the

<sup>8</sup> The difference between the mean glucose concentration before and after the injection of glucose is statistically highly significant ( $P < .01$ ).

donor dog, such as: *a*) some hemodynamic disturbance produced by the volume of fluid injected or *b*) the hypertonicity of the solution. For this purpose experiments were performed in which the donor dog received hypertonic (4%) saline instead of glucose.

The sudden hypoglycemia of *dog B* was not observed. Although a gradual decline in the blood sugar of both dogs was noted in the average curve, it was absent in 3 of the 5 donor dogs and in 2 of the 5 recipients.<sup>4</sup>

To rule out the possibility that the hypoglycemia of *dog B* might have been due to the blood of *A* per se, rather than to its insulin content, six experiments were performed in which the dogs were connected with a mesenteric-femoral instead of a pancreatic-femoral anastomosis.

Figure 4 shows that following the injection of glucose into *dog A* the blood sugar of *dog B* increased instead of decreasing.<sup>5</sup> This phenomenon is probably due to the high glucose content of the blood flowing from *dog A* to *dog B* and adds significance to the hypoglycemia observed in the dogs receiving pancreatic blood from their donors. It will be noted that, following the rise, the blood sugar of *dog B* tended to decline below the original level. This may have been due to the preceding hyperglycemia or to the tendency to hypoglycemia observed after the injection of saline for which we have no satisfactory explanation. The latter decline is, however, readily distinguishable from the sudden hypoglycemia shown in figure 1.

The possible non-specific effect of blood transfusion was further ruled out by the results of 3 experiments in which an alloxan diabetic dog was used as donor of pancreatic blood. One can see (fig. 5) that, following the injection of glucose into *dog A*, the blood sugar of *dog B* rose instead of declining, probably because of the glucose content of the pancreatic blood of *dog A* which contained no insulin.<sup>6</sup> The tolerance curve of *A* is strongly diabetic.

It appears, therefore, that the hypoglycemia noted in *dog B* receiving pancreatic blood from a normal donor cannot be explained by a non-specific effect of the injection or of the blood transfusion and was probably due to stimulation of insulin production by *dog A* following the injection of glucose.

If one compares figure 4 with figure 5 one will observe that the rise in blood sugar of a *dog B* receiving blood from the pancreatic vein of an alloxan diabetic donor (fig. 5) was much greater and lasted much longer than that of a *dog B* receiving blood from the mesenteric vein of a normal donor (fig. 4).<sup>7</sup> The difference in

<sup>4</sup> Statistical analysis showed that the decline in blood sugar concentration of *dog B* after saline injection is not significant ( $P = .6$ ). It also showed that the difference between the mean decrease in blood sugar of *dog B* after injecting glucose into *dog A* (fig. 3) and the mean decrease in blood sugar of *dog B* after injecting saline into *dog A* is significant. ( $.02 > P > .05$ ). The difference between the mean of the blood sugar concentrations reached in the 2 experiments is highly significant ( $P > .01$ ).

<sup>5</sup> The increase in blood sugar concentration is statistically significant ( $P = .05$ ).

<sup>6</sup> The difference in mean blood sugar concentration of *dog B* before and after the injection of glucose into *dog A* is highly significant ( $P > .01$ ). The difference between the mean rise in blood sugar noted in this experiment is significantly different from the mean decrease shown in fig. 3 ( $.02 > P > .05$ ).

<sup>7</sup> The difference between the mean increases in blood sugar concentration is highly significant ( $P < .01$ ).

response was too great to be explained by the higher blood sugar concentration of the alloxan diabetic donor as compared to the normal donor and suggests the possibility that the pancreas of the former may have secreted a hyperglycemic principle not masked by the simultaneous secretion of insulin. This possibility is made stronger by the fact that when *dog B* received blood from the mesenteric instead of the pancreaticoduodenal vein of an alloxan diabetic donor, its blood sugar behaved very much like that of a dog receiving blood from the mesenteric vein of a normal dog (fig. 6).<sup>8</sup>

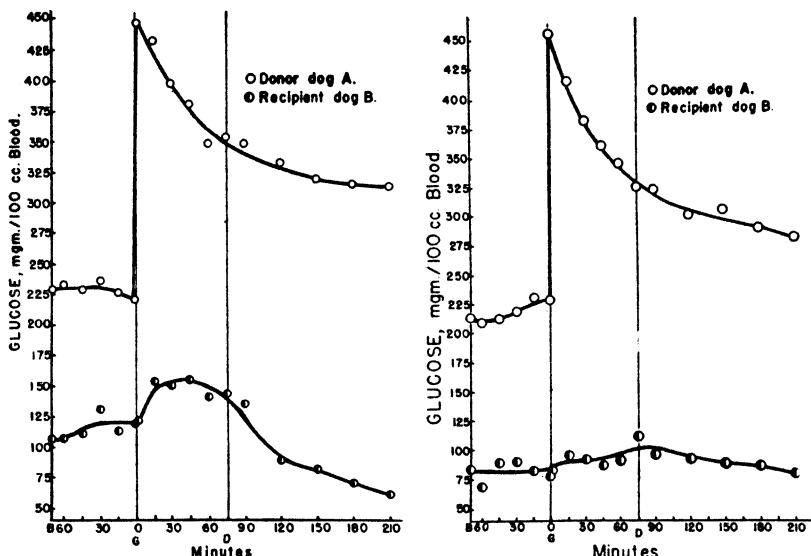


Fig. 5 (left). PANCREATIC-FEMORAL ANASTOMOSIS. Alloxan diabetic donor. Normal recipient. Average of 3 experiments.

Fig. 6 (right). MESENTERIC-FEMORAL ANASTOMOSIS. Alloxan diabetic donor. Normal recipient. Average of 2 experiments.

#### DISCUSSION

The following observations support the view that hyperglycemia stimulates insulin production: *a*) Carbohydrate tolerance is decreased after fasting or low carbohydrate diets and is restored after a carbohydrate meal (13, 14). *b*) Carbohydrate tolerance is decreased after prolonged insulin administration which probably inhibits insulin secretion (15-17). *c*) Diabetes can be produced in normal animals by feeding a high carbohydrate diet (18) or by keeping them hyperglycemic with large amounts of glucose (19). *d*) Animals can be made permanently diabetic by glucose after partial pancreatectomy (Sandmeyer's Diabetes) (20) or after partial injury to

<sup>8</sup> The rise in blood sugar concentration of *dog B* is not significant ( $P = .3$ ) whereas the difference between the mean rise observed in fig. 5 and that observed in fig. 6 is highly significant ( $P < .01$ ).

the pancreas with anterior pituitary extract (21) or alloxan (22). Apparently every time more carbohydrate is available to the animal, the insulin requirement and secretion increase. This may overtax and finally exhaust the islet cells. e) When the pancreas is protected by exogenous insulin (21, 23), or by preventing hyperglycemia with phlorizin (24), beta cell degeneration and diabetes can be prevented. f) A hypoglycemic crisis follows a carbohydrate meal in patients with hyperinsulinism (25).

Further evidence of the effects of hyperglycemia in stimulating insulin secretion was obtained by comparative measurements of blood sugar (26) or of insulin (27) concentration in the femoral and pancreaticoduodenal arteries after the injection of glucose. It was also found that hyperglycemic dog blood produces hypoglycemia in the mouse (28) (although this may be a non-specific effect of heterologous blood) and that glucose injected into the pancreatic artery reduces the systemic blood sugar (29-31). Zunz and La Barre (32) anastomosed the pancreatic vein of a *dog A* with the jugular of a *dog B* and found that the injection of glucose into *A* produced hypoglycemia in *B*. Their conclusions are based on only 3 experiments and there were no adequate blood sugar determinations preceding the injection of glucose. The effect of blood other than pancreatic was not studied. Furthermore, the hypoglycemia obtained was rather mild perhaps because the flow of blood through the vascular anastomosis was minimal, due to lack of anticoagulant and to the constriction produced by the Payr cannula. Recently Anderson and Long (33) found that the amount of insulin secreted by a perfused pancreas increases when the perfusing fluid contains glucose. Results which would not support the hypothesis that glucose stimulates insulin secretion have also been reported. Gellhorn, Feldman and Allen (34) were unable to alter the concentration of insulin in the blood by injecting glucose. Geiger and Houssay and Lewis and Foglia (quoted in 31) found that the results of Grafe and Meythaler (26, see above) were not specific as they could be obtained by using saline or urea instead of glucose. Furthermore, according to Soskin (6), the theory that insulin secretion is stimulated by hyperglycemia is not tenable in view of the experiments by himself and collaborators on the depancreatized and the hepatectomized dog. According to Soskin the hyperglycemia would inhibit the release of glucose from the liver until the blood reaches hypoglycemic levels. Not all authors concur with this point of view (Best in 6, 35, 36). Furthermore, recent evidence (37) would indicate that in man the inhibition of the release of hepatic glucose induced by hyperglycemia ceases when the blood sugar concentration is still at the relatively high value of 170 mg./100 cc.

The results of Zunz and La Barre and of other investigators quoted above were confirmed and extended by us, using the cross-circulation technique. This technique obviates the objection of using an animal of a different species for the determination of the insulin content of the blood, subjects the pancreas to a minimal trauma and maintains the recipient or insulin-detector dog nearly intact.

The evidence indicates that the hypoglycemia observed in the recipient dog following the injection of glucose into the donor is probably the result of a specific increase of insulin secretion in the donor dog brought about by the hyperglycemia. The order of magnitude of the hypoglycemia in *dog B* is similar to that observed in the normal glucose tolerance curve. It appears likely, therefore, that in the intact

normal animal the concentration of glucose in the blood regulates insulin secretion and by so doing it regulates itself. Hyperglycemia would stimulate the pancreas, the excess insulin would increase glucose utilization in the peripheral tissues (38-40) and decrease liver glycogenolysis (6). Although other mechanisms may be available to secure the regulation of the blood sugar in the depancreatized animal (41), in the intact animal the pancreas probably plays a primary rôle.

The hyperglycemia observed in the dog receiving pancreatic blood from an alloxan diabetic donor may be due to a pancreatic hyperglycemic substance similar to that found in most insulin preparations (42-44).

While this manuscript was being prepared, there appeared a paper by Sutherland and De Duve (45) showing that the *beta* cells of the islet tissue can be destroyed by alloxan without loss of the hyperglycemic factor. The hyperglycemia obtained by these authors injecting alloxan-pancreas extracts is comparable in time and duration to the hyperglycemia observed in our recipient dogs receiving pancreatic blood from an alloxanized donor. It appears, therefore, that this pancreatic principle derives from the alloxan-resistant portion of the pancreas. Sutherland and De Duve also report the interesting observation that blood destroys the hyperglycemic factor *in vitro*. Our cross-circulation experiments appear to indicate that this destruction does not occur *in vivo*, at least during the duration of the experiment. Its independence of the *beta* cells may explain why the depancreatized dog requires less insulin than the dog in which diabetes has been produced not by removal of the organ, but by injury to the *beta* cells with alloxan or anterior pituitary extract (46). It may also help explain why a totally depancreatized man requires less insulin than a moderately severe diabetic (47). Experiments designed to demonstrate the presence of a hyperglycemic principle in the pancreas of alloxan diabetic dogs are in progress.

#### SUMMARY

In 35 cross-circulation experiments, anastomoses were made between the pancreaticoduodenal or the mesenteric vein of a donor *dog A* and the femoral vein of a recipient *dog B*. A return circulation was obtained by establishing a limited flow of blood between the femoral artery of *dog B* and the femoral vein of *dog A*.

The blood sugar of a dog receiving pancreatic blood from a normal donor injected with glucose decreases sharply reaching a minimum in 30 to 60 minutes and gradually returning toward normal about 2 hours after the anastomosis has been disconnected. This sharp decrease is similar to the hypoglycemic phase of the normal glucose tolerance curve. Both phenomena are consistent with the hypothesis that a rise in blood sugar stimulates the secretion of insulin and by so doing regulates itself.

When saline is injected instead of glucose the glycemia of *dog B* does not decrease significantly. When the transfusing blood derives from the mesenteric vein of a normal donor the blood sugar of the recipient dog increases instead of decreasing. The increase in the blood sugar concentration of the recipient dog is even more marked and sustained if the anastomosis is made with the pancreatic but not with the mesenteric vein of an alloxan diabetic donor. The latter hyperglycemia sug-

gests that the pancreatic blood of the alloxan diabetic dog contains a hyperglycemic substance produced by the alloxan resistant portion of the pancreas and active *in vivo*.

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# COMPARATIVE EFFECT OF INTRAVENOUS GLUCOSE AND ADRENALIN ON BLOOD FLOW, OXYGEN UTILIZATION AND GLUCOSE RETENTION BY HIND LEG TISSUES OF ANESTHETIZED CATS

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**I**N A recent report (1) it was shown that although the carbohydrate plethora of an adrenalin hyperglycemia is invariably accompanied by increased transfer of sugar from blood to peripheral (hind leg) tissues, this increase in tissue sugar uptake or retention was usually accompanied by a decrease rather than an increase in tissue oxygen consumption.

Insofar as a specific calorigenic action of adrenalin itself might be involved, this result was not unexpected since we have been repeatedly unable to detect a locus for it in peripheral tissue of intact animals (2-4) and there is considerable evidence, too extensive to be documented here, that it is abrogated by hepatectomy or evisceration.

Insofar, however, as the carbohydrate plethora itself might be involved, the result was unexpected since the locus of its specific dynamic action is presumably in the peripheral tissues themselves; it not only occurs after hepatectomy or evisceration (5, 6) but apparently is even intensified thereby (7).

If the only effect on oxygen consumption in the experiments referred to had been a depression, it might have been concluded that adrenalin directly suppressed the specific dynamic action of the very carbohydrate plethora evoked by it. The suppression of oxygen consumption of the leg tissues was, however, not invariable; there were enough instances in which it increased to suggest that whichever effect occurred must depend on some indirect effect of the intravenously injected adrenalin and it was suggested that this might be its effect on blood flow within the limb. Whenever this was decreased (which apparently could only result from such intense local vasoconstriction as to offset the effect of a presumably elevated arterial blood pressure) oxygen consumption was decreased also. When blood flow through the leg was increased it was assumed this might occur in two ways: 1) sufficient elevation of blood pressure to drive an increased amount of blood through still patent channels and in spite of extensive local vasoconstriction; and 2) actual local vasodilatation if the effective adrenalin concentration reaching the leg was of a minimally effective magnitude. In (1) the oxygen supply to and its utilization by the leg tissues would be as effectively curtailed as in those experiments in which the blood flow was actually diminished; in (2) oxygen supply would be and its utilization presumably could be increased. This, at least, seemed to provide a possible explanation for the finding that: a) oxygen consumption always decreased if blood flow decreased; b) never increased unless blood flow increased; and c) might at times be decreased even though blood flow was increased.

This interpretation is based on assumptions which seem consonant with generally accepted premises as to the vascular readjustments effected by adrenalin. It was obviously desirable, however, to have direct measurement under the conditions of these experiments of the actual change of blood pressure and of the degree of vaso-

constriction or dilatation (plethysmograph) coincident with the measured oxygen consumption by and flow rate through the leg tissues. In addition, it was desired to compare the effects of adrenalin on these variables with those accompanying a hyperglycemia of approximately equal magnitude produced by glucose injection alone. For although there are scattered observations in the literature as to tissue glucose uptake resulting from a glucose hyperglycemia, there are none known to us which permit a quantitative comparison of this with the effect of an adrenalin hyperglycemia of equal degree. Nor do we know of any measurement of the effect of glucose injections of this magnitude on arterial blood pressure, or blood flow and oxygen utilization by peripheral (leg) tissues.

It is the purpose of this report, then, to compare the effect on arterial blood pressure and leg volume, and the change in blood flow, sugar retention and oxygen consumption of the leg which result from: *a*) intravenously injected adrenalin; and *b*) glucose injected intravenously in such amounts as to effect a comparable hyperglycemia.

#### PROCEDURE

All of the work was done on cats anesthetized with dial-urethane (Ciba). Intravenous injection of adrenalin or glucose was by way of the cannulated left jugular vein. Arterial blood pressure was recorded from the right carotid by means of a Hürthle membrane manometer. Heparin was administered intravenously to prevent clotting.

Arterial blood samples for determination of arterial glucose, oxygen and hemoglobin concentrations were obtained from a cannula in the right iliac artery.

Venous blood from the left hind leg was obtained from a special cannula in the right iliac vein into which flowed all the blood from the left hind leg when the vena cava was clamped just above its origin. As the blood entered this cannula it was kept at a fixed mark on a vertical side arm by withdrawal into a tightly fitting syringe the rate of filling of which was timed with a stopwatch, thus measuring the rate of blood flow from the left leg. Most of this blood would be immediately reinjected and only enough retained in the syringe for determination of venous glucose, oxygen and hemoglobin concentrations. These values and those of a simultaneously obtained arterial blood sample provided the arterio-venous differences in concentrations which, together with the measured rate of blood flow, permitted calculation of the sugar retained and oxygen used by the tissues of the left hind leg per unit time.

After these preparations were complete the left hind leg was enclosed in a plethysmograph fitting as high up toward the hip as possible. An air-tight seal with the shaved skin was uniformly easily obtained with Unna paste. The plethysmograph was insulated from sudden variations of room temperature by thick wrapping with cotton batting. Variation of leg volume was transmitted by air to a Marey tambour and recorded on the smoked kymograph paper synchronously with the arterial blood pressure.

Adrenalin was employed in only one dosage, 0.004 mg/kg. of body weight per minute in one cubic centimeter; to obtain this, proper dilution of Parke-Davis 1:1000 adrenalin chloride solution was made with isotonic NaCl solution immediately before use. Injection was by a hand-operated syringe into the jugular vein and timed with

a stopwatch at a rate as nearly as possible of 1 cc/min. for 5 minutes; it was begun immediately after securing a pair of simultaneous normal arterial and venous blood samples; and its immediate effect was determined by another pair of blood samples taken during the terminal portion of the 5th minute of injection. After-effects were determined by samples obtained 5, 15 and 30 minutes after the end of the 5-minute injection period.

Glucose hyperglycemia was desired to be as nearly as possible equal to that induced by the adrenalin; for the rate of adrenalin injection used here had been found in previous work (1) to be an average increase of about 85 mg. per cent. Preliminary trial indicated that an increased blood sugar level of approximately this amount would be caused by intravenous injection of 0.25 gm. of glucose per kg. of body weight in 5 minutes (0.05 gm/kg/min. for 5 min.). For reasons not significant here, this amount was dissolved in 2.5 cc. of distilled water and injected, as was the adrenalin, into the jugular vein by a hand-operated syringe at the rate of 0.5 cc/min. for 5 minutes. Blood samples were taken as described for adrenalin.

Blood sugar was determined by the method of Hagedorn and Jensen; hemoglobin determinations for assessment of change of blood hydration were by the colorimetric method of Cohen and Smith; blood oxygen content was obtained by the manometric method of Van Slyke and Neill; careful precaution was taken to prevent alteration of gas content of the blood samples during collection and while awaiting analysis.

#### RESULTS

The data to be presented were derived from 25 experiments with adrenalin and an equal number with glucose injected at the rates and in the quantities described above.

*Blood hydration.* As in our previous work, and as have others, we find that venous blood from the leg is consistently (and in this work invariably) more concentrated than the arterial blood going to it as measured by the change in hemoglobin concentration and expressed as oxygen capacity. Also, as before, the degree of concentration varies greatly from one experiment to another so it is not surprising to find that the average for the 50 normals of this series is somewhat different (higher) than reported previously by us; i.e., the average oxygen capacity of venous blood in this series was 0.81 vol. per cent greater than arterial. But, again, in conformity with previous findings, intravenously administered adrenalin affects this very little and in the present instance not at all, the difference being exactly the same at the end of the 5-minute injection period. Nor did glucose infusion change this blood-tissue water exchange to any marked degree, merely diminishing it slightly so that the oxygen capacity of venous blood was only 0.74 volume per cent greater than arterial at the end of the 5-minute intravenous injection. When, later on, the effect of the glucose infusion on total leg volume is considered this evidence of an only slightly diminished loss of water from arterial blood to the tissues will have to be reconsidered.

The oxygen contents of venous blood samples have in all cases been corrected for the concurrently indicated change in blood hydration in this as in our previous work. For there is apparently no reason to doubt that, since the bulk of the oxygen

is in the corpuscles, this dehydration of the blood as it passes through the leg makes the apparent oxygen content of venous too high in comparison with that of arterial blood.

Blood sugar, however, now appears to us to be in a different category. Whereas, in our previous work venous blood values were similarly corrected for the change in hydration, it now seems that until there is definite evidence to the contrary, it is safer to assume that the water leaving the blood will carry small molecules with it without any change in concentration. Whether applied or not this correction makes no difference qualitatively and in most instances little quantitatively.

*Hyperglycemia.* Intravenous injection of adrenalin at the rate and for the time employed here (0.004 mg/kg/min. for 5 min.) produced an increase in arterial blood sugar level or a hyperglycemia of 77 mg. per cent as the average of the 25 experiments. This checks well with our previous work in which the average of 14 injections at this same rate was 85 mg. per cent.

Since it was desired to raise the blood sugar level as nearly as possible an exactly equal amount by intravenous glucose infusion, the preliminary selection of 0.05 gm/kg/min. for 5 minutes as the rate of administration proved to be singularly appropriate; the average elevation of arterial blood sugar level thus induced at the end of the 5-minute infusion period for the 25 experiments was 82 mg. per cent—a remarkably close approximation to the effect produced by the injection of adrenalin.

During the half hour following the termination of the 5-minute injection period the decline of the blood sugar level was more rapid in the glucose than the adrenalin series (fig. 1); at the time intervals, 5, 15 and 30 minutes after injection, blood sugar following glucose injection was 58, 47 and 11 mg. per cent above normal; the corresponding values after adrenalin are 75, 57 and 51 mg. per cent. Whatever the cause of this difference, it cannot be a reduced uptake of sugar by the peripheral tissues following adrenalin since it will be shown below that, if anything, this is then greater than after pure glucose administration. One thinks of possible inhibition of insulin secretion by the injected adrenalin and a retarded reconversion of blood sugar to glycogen; but although such an inhibitory effect has been reported, a contrary action has also been claimed for it, so that the effect observed here will have to await explanation.

*Sugar uptake.* Without exception, arterial blood sugar level was always higher than that in venous blood leaving the hind leg; the average arterio-venous (A-V) difference for the 50 normals of this group of experiments was 21 mg. per cent; this represented an average rate of glucose retention by the leg tissues of 3.22 mg. per minute.

The adrenalin and glucose hyperglycemias (+77 and +82 mg. % respectively) were accompanied by an increase in this transfer of glucose from blood to leg tissues (fig. 1): at the end of the 5-minute injection of adrenalin, the A-V difference increased to 45 mg. per cent, and glucose retention to 7.07 mg. per minute, or an increase of the latter to 220 per cent of normal; the corresponding figures at the end of the 5-minute glucose injection are 31 mg. per cent, 6.05 mg. per minute and 188 per cent of normal. Thus, if anything, the slightly lesser adrenalin hyperglycemia is accompanied by a greater increase in the transfer of glucose to these peripheral leg tissues than is the slightly greater one resulting from glucose administration. Per-

haps there is nothing significant in this, but at least it demonstrates that adrenalin has no specific inhibitory effect on the uptake of blood sugar by the peripheral tissues during the development of its hyperglycemic effect.

This latter is also true for the most part during the half-hour post-injection period. For the intervals 5, 15 and 30 minutes after the end of injection the figures are: adrenalin series: A-V difference, 28, 21 and 24 mg. per cent; glucose uptake, 4.37,

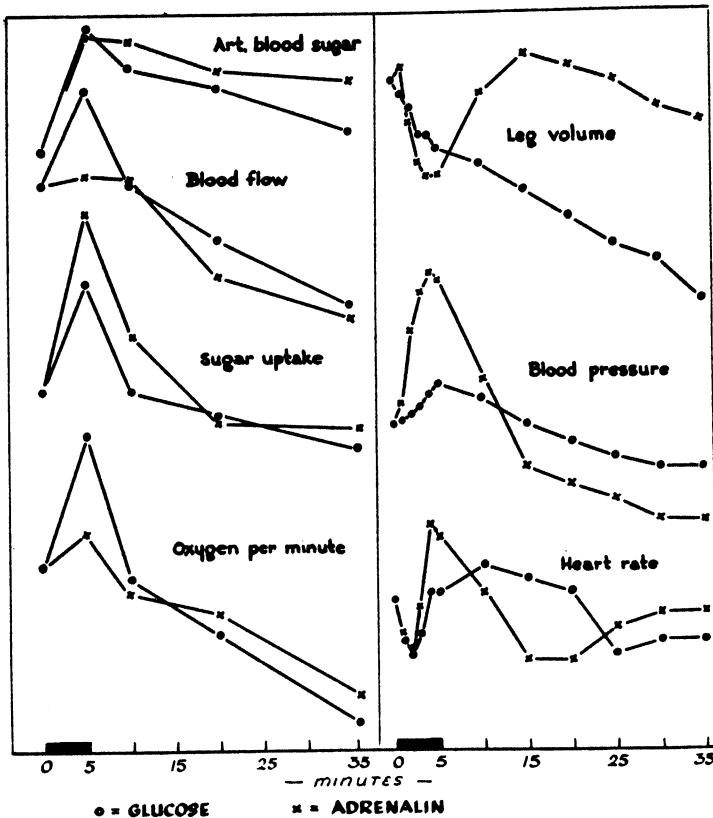


Fig. 1. EFFECT OF 5-MINUTE INTRAVENOUS INJECTION of adrenalin (0.004 mg/kg/min.) and of glucose (0.05 gm/kg/min.) on the circulation in and glucose retention and oxygen utilization by the hind leg tissues of dial-urethane (Ciba) anesthetized cats. For description see text.

2.33 and 2.26 mg. per min.; per cent of normal, 136, 72 and 70; glucose series: A-V difference, 22, 22 and 20 mg. per cent; glucose uptake, 3.37, 2.84 and 2.00 mg. per min.; per cent of normal, 105, 88 and 62. Thus, except for the interval 15 minutes after injection, the transfer of sugar from blood to leg tissues continues to be greater after adrenalin than after glucose administration (fig. 1).

During this 30-minute after-injection period the blood sugar level is declining, but more rapidly after glucose than after adrenalin injection; and this better-sustained arterial sugar concentration after adrenalin might be a factor in maintaining

the just-mentioned greater transfer from blood to tissues which continues during this half-hour post-adrenalin as compared with the post-glucose period. On the other hand it could have been anticipated that in both series as soon as the hyperglycemas began to decline from their highest levels, the transfer of glucose would reverse and be from tissue to blood. This would be particularly expected if the increased blood-to-tissue transfer at the height of the elevated blood sugar levels is regarded as merely a physical equilibrium involving no increase in glucose metabolism or utilization. Whether or not it is of any significance in this connection it may be remarked, however, that such a reversal was never seen during the post-injection periods of declining arterial blood sugar level; the A-V differences were always positive and the glucose transfer always from blood to tissue, even though the total transfer per minute had fallen, 15 and 30 minutes after injection, to only 88 and 62 per cent (glucose series) and 72 and 70 per cent (adrenalin series) of normal. This ultimate reduction to less than normal sugar uptake by the tissues may be linked with the greatly reduced blood flow which occurred at this same time.

*Blood flow.* The average rate of blood flow through the hind leg for the 50 normal determinations was 15.3 cc/min.; this agrees well with a previous average (1) of 16.4 cc/min. for 212 measurements of normal flow rate.

*Adrenalin effect.* At the end of the 5-minute intravenous injection of adrenalin at the rate of 0.004 mg/kg/min., flow rate was increased only 3 per cent for the average of the entire series of 25 experiments (fig. 1); this is approximately of the same magnitude as, though somewhat less than the increase of 8 per cent as the average of the 44 experiments of the previous work, just referred to, in which adrenalin was injected at this same rate.

The value of such confirmation resides chiefly in the fact that the effect on blood flow of adrenalin injected intravenously at the rate used here is, in individual instances, unpredictable; it may be increased or decreased. Further reassurance that the effects obtained are not entirely capricious but are following some pattern imposed by the experimental conditions, such perhaps as varying depth of anesthesia or other factor or factors as yet unsuspected, is derived from the relatively constant proportion of decreases to increases: in the present work these were 36 and 64, in the former, 43 and 57 per cent, respectively.

Some insight into the mechanism of this variability of response is afforded by the blood pressure, heart rate and leg plethysmograph records which were concurrently obtained and the averages of which for the entire series are shown in figure 1.

Comparing those experiments in which blood flow was increased, with those in which it was decreased at the termination of the 5-minute adrenalin injection period, it is found that the plethysmographic change, measured as the deviation in millimeters from the normal base-line, is practically the same in both groups. The change, as the average per cent of normal, for each minute of the 5-minute injection was for the 16 experiments in which blood flow was increased: 118, 65, 50, 42 and 41; and for the 9 experiments in which blood flow was decreased: 104, 76, 48, 45 and 57; thus, what difference there is in the average reaction of the two groups, indicates a slightly greater decrease of leg volume (vasoconstriction) when blood flow was increased.

The cause of this increased blood flow in spite of slightly greater reduction in

leg volume is probably found in the average change in arterial blood pressure of the two groups. The averages for each minute of the 5-minute injection period were, when blood flow increased: 8, 32, 46, 56 and 54 mm. Hg above normal; and when blood flow decreased: 3, 19, 25, 3 and 1 mm. Hg above normal; i.e., in the first group, blood pressure rises progressively during the first four minutes of injection and then steadies at a high level, 51 to 54 mm. Hg above normal; whereas, in the other, after reaching a maximum 25 mm. above normal during the third minute of injection it drops rapidly almost back to normal.

The behavior of heart rate is also interesting during this 5-minute injection period. The averages for each minute for the group in which blood flow was increased were, as deviations in beats per minute from normal: -2, -9, -6, +11 and +14; for the group in which blood flow decreased: -12, -6, +8, +9 and zero; i.e., in both groups there is an initial cardiac inhibition as the blood pressure rise gets under way but this is more alert and severe in the latter group; in this group, too, the secondary tendency to increase in rate is quickly and effectively checked so that the rate is finally held at normal.

In summary, two groups of effects during the last minute of a 5-minute intravenous injection of adrenalin are correlated as follows: in one group blood flow through the leg is increased; at this time arterial blood pressure is markedly elevated, heart rate is above normal and leg volume is decreased; in the other group blood flow in the leg is decreased, blood pressure and heart rate are either practically or entirely unchanged and there is slightly less decrease in leg volume.

Not knowing of any previous results of just this nature, or, therefore of attempts to explain them, an hypothesis may tentatively be advanced to account for them, or at least serve as a guide to further work. We will assume that the ultimate cardiac and smooth muscle responses, cardiac acceleration and peripheral vasoconstriction, are probably not markedly variable for a fixed rate of intravenous adrenalin administration, and probably not markedly affected by chance variations of the fairly fixed conditions of these experiments. It seems altogether probable, however, that the nervous regulatory mechanisms, such as the moderator reflexes, might be significantly affected in sensitivity by such variations in duration or intensity of narcosis as might inadvertently occur from time to time in the same animal or especially as between different preparations. If these were not depressed, any tendency of blood pressure to rise or heart rate to increase as a result of the peripheral action of adrenalin would be effectively countered by reflex cardiac and vasoconstrictor inhibition; the vasoconstrictor decrease in leg volume would be moderated and the cardiac acceleration and blood pressure rise, as a result of 'over-shooting', might for a time be completely abrogated. These are the conditions observed in the group of experiments in which as a consequence of the remaining peripheral vasoconstriction and the failure of arterial pressure to be elevated, the amount of blood driven through the leg per minute is diminished. On the other hand if the moderator reflex centers were sufficiently depressed, blood pressure and heart rate would increase with less effective counteraction and although peripheral vasoconstriction would remain even somewhat more intense than in the first group, the sizable elevation of pressure could drive even more blood through still patent channels so as to augment it even above normal.

This matter is dwelt upon at this length in a report whose chief interest is in the effect of adrenalin on tissue oxygen consumption because as will be seen below, there is good evidence that at least in large part its effect on this is secondary to that on blood flow. It is also a useful corrective for what seems to be the general opinion that the effect of adrenalin is either one thing or another; our experience has been that at least in so far as peripheral blood flow and oxygen consumption are concerned the effect under the usual experimental conditions is qualitatively variable precisely, as we suppose, because of the complex underlying interrelationships just reviewed.

*Glucose effect on blood flow.* Glucose administered intravenously in the amount used here, 0.05 gm./kg./min. for 5 minutes, increased blood flow in the hind leg during the terminal part of the last minute of injection from the average normal of 15.3 to 19.5 cc. per minute or 28 per cent as the average of the entire series of 25 experiments (fig. 1). By contrast, it will be recalled that the corresponding total average for the effect of adrenalin was the very slight increase of only 3 per cent. Also in contrast to the effect of adrenalin, that of glucose was more constant in that flow rate was increased in 20 of the 25 experiments; of the remaining five there was practically no change in two and for the three in which there was an actual decrease the average of -2.5 cc/min. was much less than the average decrease of 7.9 cc/min. for the nine of the adrenalin series in which this occurred. It may be said, then, that the effect of glucose administered intravenously in the amount used here on blood flow in the tissues of the leg is predominantly to increase it and to a rather marked degree.

The mechanism of this increase can be deduced only in part from the concomitant records of blood pressure and leg volume (fig. 1). Such change in blood pressure as occurred would contribute to it, but perhaps could not be considered its only cause for it can be seen that the average increase is only slight, +11 mm. Hg, as compared with the large increase of 44 mm. induced by adrenalin. As to why blood pressure should be increased at all, it is probable that the small amount of injected fluid, 0.5 cc/min. for 5 minutes, was without effect; nor, apparently, did the heart contribute to it, since after an initial depression of rate as the blood pressure rise was getting under way it returned only to the pre-existing normal as the injection ended.

It is seen however that during the 5-minute injection period, leg volume is progressively decreased and to almost as great an extent as by adrenalin. It is unlikely that with glucose this decrease would be due to vasoconstriction. In fact, the contrary is suggested by the evidently active moderator reflex and cardiac inhibition which obtains at least during most of the injection period. If there were a corresponding reflex constrictor antagonism of any degree or even if there were no vasomotor effect in the leg at all, the decrease in leg volume would have to be due to osmotic abstraction of water into the hypertonic, sugar-enriched blood. This seems theoretically likely and would explain the rise in arterial blood pressure as a result of hydremic plethora. In fact, in spite of contrary evidence this is the only mechanism we can think of; but it must be recalled in this connection, as mentioned in a previous section on blood hydration, that all blood hemoglobin determinations indicated passage of water from blood to tissues; i.e., venous blood from the leg always had a higher hemoglobin concentration than the arterial blood going to it; and this trend was never reversed, but only reduced slightly below the normal value.

by injection of glucose. It is therefore impossible from these data to account satisfactorily for the marked and almost unexceptional increase of blood flow in the leg which resulted from the injection of glucose; an alternative explanation based on the local vasodilator action of increased tissue metabolic rate will be more appropriately presented in a later section in connection with the effects on oxygen consumption by the leg.

*After effects.* It is seen (fig. 1) that five minutes after the injections were ended blood flow in both the adrenalin and glucose series had returned to the pre-existing normal value. Thereafter decline is continuous and almost uniform but slightly faster in the adrenalin series to final values approximately 60 per cent of normal 30 minutes after injection. This is similar to the decline we have always observed during observation periods of this duration even in controls without any injection whatever and is probably attributable merely to the deteriorating condition of the animal as a result of the frequent blood sampling and other trauma. It can be seen that blood pressure shows a similar decline and heart rate is for the most part failing.

The most interesting event during this half-hour post-injection period is the contrasting behavior of leg volume in the adrenalin and glucose series. After adrenalin the volume returns to, and slightly above normal shortly after the injection has stopped and falls below normal only toward the end of the half-hour period. After glucose, on the other hand, the sharp initial decrease in volume during the injection is followed by a continued decline at a lesser but steady and unbroken rate until the end. If decrease of leg volume in this case is due to osmotic attraction of water from tissues to blood, this evidence would indicate a rather slower equilibrium than might have been anticipated; if it is not due to this (and see objections above) we have no explanation for it.

*Blood pressure, heart rate and leg plethysmograph.* The only interest these have for the present is the insight they might afford as to the mechanism of blood flow change in the leg. As such they have been used in the preceding section on *Blood flow* and will receive no further consideration here.

*Oxygen utilization by the leg tissues.* It may be recalled that the impetus behind this work was a previous observation that although glucose uptake by leg tissues was invariably largely increased during an adrenalin hyperglycemia, their oxygen utilization was not, but appeared to be related rather to the effect of adrenalin on the circulation in the leg. Since there was existing evidence that a carbohydrate plethora from intravenously administered glucose could by itself stimulate the oxygen utilization of peripheral tissues (specific dynamic action) it became desirable to know whether the increase in the amount of glucose getting into the tissues to serve as stimulus was less when the hyperglycemia owed its origin to adrenalin, than when it resulted from glucose administration alone; and also whether oxygen utilization by the leg tissues following glucose administration would have any relation to altered blood flow as after adrenalin.

The first of the above questions has been answered in a previous section where (and also see fig. 1) it was shown that from almost exactly equal hyperglycemic levels the sugar uptake by the leg tissues was actually greater (220% of normal) when the hyperglycemia was due to adrenalin than when due to glucose alone (188% of normal). Therefore from the point of view of tissue carbohydrate plethora and stimulus, alone,

glucose specific dynamic action should be greater in the adrenalin than in the glucose series.

It will now be seen that not this, but its reverse is true. For each group as a whole (average of 25 experiments each) oxygen consumption was increased at the end of the 5-minute intravenous injection of adrenalin only 5 per cent; at the end of the 5-minute intravenous glucose injection the increase is 19 per cent; i.e., the considerably greater increase in sugar uptake (adrenalin) is associated with a very small increase in oxygen consumption, whereas the lesser increase in tissue sugar retention after glucose administration is accompanied by a marked increase in oxygen utilization.

Correlation of oxygen utilization with blood flow, however, corroborates our previous observation and is, for the total averages, as follows: glucose hyperglycemia is accompanied by an increased leg blood flow of 28 per cent and leg oxygen consumption of 19 per cent; and for the adrenalin hyperglycemia the corresponding figures are 3 and 5 per cent, respectively.

It will be recalled that neither adrenalin nor glucose administration were invariable in their effects on leg blood flow. With adrenalin, flow rate was increased in 16 and decreased in 9 experiments; and for the glucose series the corresponding figures were 20 and 5, respectively. In all but one of the 16 in which adrenalin increased flow rate, oxygen utilization increased also; and in only 2 of the 9 in which flow rate decreased did oxygen consumption fail to decrease also. Likewise in the 20 experiments in which flow rate increased as a result of glucose administration, oxygen consumption failed to increase in only 5 and these were rather in the range of no significant change than of definite decreases; and in the 5 experiments in which flow rate decreased with glucose injection, oxygen utilization also decreased in 3, with, again, the 2 exceptions being rather in the class of no marked change than of definite increases.

This correlation between blood flow, rather than glucose uptake, and leg tissue oxygen consumption is further exemplified by the post-injection trends of these three variables; as shown graphically in figure 1 the rates of decline of flow rate and oxygen utilization are very similar in these deteriorating animals while the course of change in sugar uptake is quite different.

It seems necessary to conclude therefore that the failure of an adrenalin hyperglycemia to be accompanied more generally and to a greater degree by increased oxygen utilization by peripheral tissues than is hyperglycemia resulting from glucose injection, is not due to a reduction by adrenalin of the amount of stimulating blood sugar which reaches and is retained by the tissues. Rather, what seems determinant for oxygen consumption is blood flow. Both as a rule are decreased and increased together; and since the effect of glucose alone is to cause a much greater average increase in leg blood flow it is also accompanied by a much greater average increase in leg tissue oxygen utilization.

The actual mechanism underlying these correlations may tentatively be suggested as follows: increased influx of glucose into the tissues of the leg stimulates their respiratory metabolism, i.e., has its usual specific dynamic action. Increased carbon dioxide formation could cause local vasodilatation thus providing a major factor for the increased blood flow following glucose infusion as well as an increase in the oxygen supply to the tissues commensurate with their new metabolic state.

If adrenalin in the amount used here is present in the blood reaching the leg tissues, vasoconstriction of varying degree depending on local conditions may be safely presupposed at least for these quiescent anesthetized animals; the effect might be quite different under conditions of normal and especially of emergency activity. Arteriolar constriction could be sufficient to block the passage of red cells but still permit a flow of plasma of high glucose content, the plasma skimming observed by Hartman *et al.*, (8). Passage of glucose into the tissues might then be equal for the short-time period used here to that in the previous instance, but oxygen for its oxidation would not reach the tissues at the same time. Such would be the most extreme case; but since this local reaction in the leg would conceivably be of almost any degree of intensity and since arterial perfusion pressure following general intravenous administration may also be increased over wide limits the net result as has been seen will necessarily be variable. And the only generalization that could safely be made would be that blood flow and oxygen utilization under the influence of glucose alone would very likely be more generally increased and to a greater degree than when adrenalin is present at the same time. And this could be used as a statement of the main conclusion to be derived from this work.

It may be emphasized again that this conclusion is intended to apply only to the conditions of these experiments. If adrenalin were employed in minimally effective and vasodilator or maximally constrictor dosages or if instead of being quiescent, anesthetized and progressively moribund the animals had been in more normal physiological condition, the results might be expected, from generally accepted premises, to be quite different.

#### SUMMARY

Intravenous injection into dial-urethanized cats of adrenalin at the rate of 0.004 mg/kg/min. for 5 minutes and of glucose at the rate of 0.05 gm/kg/min. for 5 minutes produced hyperglycemas of marked average similarity: +77 and +82 mg. per cent respectively. From these hyperglycemic blood sugar levels glucose transfer from blood to leg tissues was increased 120 and 88 per cent by adrenalin and glucose, respectively; so that adrenalin at least did not inhibit this effect.

The average effect of adrenalin on blood flow through the leg was to increase it 3 per cent; of glucose, 28 per cent. Both might in individual instances cause either an increase or a decrease; but after glucose the increases were more common and of greater degree. Oxygen utilization by the leg tissues followed the change in blood flow rather than of glucose uptake; thus, glucose injection increased it 19 per cent and adrenalin injection only 5 per cent.

Records of arterial blood pressure, heart rate and leg volume (plethysmograph) were obtained and used in an effort to account for the observed changes in leg blood flow.

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# THYROID ACTIVITY AND RESISTANCE TO HISTAMINE- INDUCED PEPTIC ULCER AND TO ACUTE HISTAMINE POISONING<sup>1</sup>

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**I**T has been recognized for some time that a relationship exists between the functional state of the thyroid gland and the secretory and motor activity of the stomach. One of the early observations was made by Friis Moller in 1914 (1) who noted that anacidity was common in patients with hyperthyroid disease. The feeding of thyroid substance to dogs (2) and rabbits (3) has been reported to diminish gastric secretion. Gastric motor activity appears to be augmented in dogs fed thyroid substance (4) and this effect is not altered by vagotomy (5). Truesdell (2) working with Pavlov pouch dogs observed marked reduction of acid secretion after feeding desiccated thyroid and opined that some disturbance of the nervous control of gastric secretion had occurred. Several thyro-gastric relationships have been described in the clinical literature. Increased incidence of peptic ulcer in connection with hypothyroidism and the converse have been noted (1) as well as successful therapeutic use of desiccated thyroid in hyperacidity and peptic ulcer (6). On the other hand Friedenwald and Morrison state (7): "The gastric acidity is usually diminished in hypothyroidism more frequently than in hyperthyroidism."

It was decided to investigate the effect of alterations of thyroid activity upon the gastric response to histamine as measured by the experimental production of peptic ulcer.

## METHODS

Seventy-five male guinea pigs were used in this investigation. A commercial ration, fortified with ascorbic acid, and water were allowed *ad libitum* and the animals were kept in a room maintained at  $27^{\circ} \pm 1^{\circ}\text{C}$ .

Basal metabolic rates are expressed as Cal/hr/kg.  $\frac{3}{4}$  and were determined in a modified Haldane apparatus (8) just prior to histamine treatment.

Since the metabolic rates were determined on groups of 6 animals, the presence of an atypical individual, e.g. an animal with incomplete thyroidectomy, might be reflected as a small error in the group average. Body weight records revealed, however, that all animals reacted, grossly at least, in a manner typical of the altered thyroid function.

Received for publication February 28, 1949.

<sup>1</sup> This investigation was supported in part by a grant from the Department of the Navy, Office of Naval Research.

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Histamine was administered intramuscularly in the form of a suspension in beeswax and mineral oil<sup>8</sup> (9, 10). It was given in two equally divided doses totaling 1 mg./100 gm. of body weight per 24 hours. When benadryl (beta dimethylamino-ethyl-benz-hydryl ether hydrochloride) was used to protect the animals against the acute toxic effects of histamine, it was administered intraperitoneally, 30 minutes before each administration of the histamine suspension, in a dosage of 1 mg./100 gm. of body weight. This dosage of benadryl has been shown to be more than adequate to protect guinea pigs against 5 or 6 times the lethal dose<sub>100</sub> of histamine administered intravenously (11).

Natural crystalline thyroxine (Squibb) was given in a dosage of 0.1 mg. subcutaneously every other day. Desiccated thyroid was given orally in doses of 30 mg. daily and carefully washed down with water. Thiouracil was given *ad libitum* in the form of a 0.1 per cent solution in place of drinking water. Thyroidectomy was performed under ether anesthesia and the gland totally extirpated with preservation of the parathyroids. The animals were permitted a six-week recovery period before the B.M.R. was measured and histamine treatment begun.

In all cases medication was continued during the histamine administration. The end point of the experiment was death, due to either perforation of a peptic ulcer with consequent general peritonitis or acute histamine poisoning. Those animals failing to expire after 21 days of histamine administration and which, on post-mortem examination, presented no evidence of peptic ulcer were considered 'histamine resistant'.

Time was measured in hours from the first histamine injection to the time of death. All animals were autopsied and microscopic examination made of the organs of animals of each group. During the course of the work the animals were examined at such intervals that a maximum delay of four hours in noting death was possible but, in general, it was less than two hours.

Various experimental procedures were carried out on groups of animals as follows:

*Group I: 9 animals.* Average body weight 460 gm. Basal metabolic rate was measured and the histamine injections begun.

*Group II: 8 animals.* Average body weight 451 gm. These animals were treated like those of *Group I* except that benadryl was administered 30 minutes before each injection of histamine suspension.

*Group III: 11 animals.* Average body weight 490 gm. These animals were thyroidectomized, allowed a 6-week recovery period and then treated like *Group I*.

*Group IV: 11 animals.* Average body weight 484 gm. These animals were premedicated for 6 weeks with thiouracil and then treated like *Group I*.

*Group V: 12 animals.* Average body weight 504 gm. Natural crystalline thyroxin as described was given for 6 weeks before histamine injections were begun.

*Group VI: 12 animals.* Average body weight 440 gm. This group was treated like *Group V* except that benadryl protection was given as described for *Group II*.

*Group VII: 6 animals.* Average body weight 430 gm. These animals were given desiccated thyroid as described for 6 weeks before histamine injections were begun.

*Group VIII: 6 animals.* Average body weight 510 gm. These animals, too, were given desiccated thyroid and treated like those of *Group VII* but in addition were given benadryl protection as described.

<sup>8</sup> Histamine-Beeswax was a gift from Hoffman-LaRoche, Inc., Nutley, N. J.

## RESULTS

Table I summarizes the results obtained with *Groups I* to *IV*. *Groups V* and *VII* were both made hyperthyroid and may be considered jointly. The metabolic rates for *Group V* (thyroxin) and *Group VII* (desiccated thyroid) were 31 per cent and 17 per cent, respectively, above the value for *Group I*. Every animal in both groups died within half an hour after the first histamine injection. These animals in all cases exhibited the typical manifestations of acute histamine poisoning and, of course, no evidence of peptic ulcer. This response to the suspension of histamine, which produced no acute reactions in most normal and hypothyroid animals, was so dramatic and definite that it was decided to attempt protection of later hyperthyroid groups with benadryl.

*Groups VI* and *VIII* may also be considered together. These hyperthyroid animals were given benadryl in the hope that fatal reactions to histamine might be prevented and thus permit the usual observation of the development of perforated peptic ulcers. The survival time was increased but the animals, nevertheless, died before the typical ulcer endpoint was reached. All of the animals succumbed to acute histamine poisoning shortly after one of the first seven injections of histamine, i.e. within 74 hours. Of the 18 animals in *Groups VI* and *VIII*, 9 died within 12 hours after histamine dosage was begun, 5 more died in the next 48 hours and the last 4 animals died at 74 hours. Among the 4 succumbing to the seventh injection only two had nonperforated peptic ulcers. The remaining 16 animals failed to exhibit any signs of ulcer.

## STATISTICAL ANALYSIS

Application of the 't' test and 'Student's' chart to the data on survival times yields the following comparisons among the first four groups:

GROUP	IV	III	II
I	t = 0.79 p = 0.45	t = 3.43 p = 0.004	t = 0.706 p = 0.48
II	t = 0.044 p = 0.97	t = 2.251 p = 0.040	
III	t = 2.32 p = 0.033		

Thus there are significant differences between groups I and III, II and III and III and IV. Since the animals in *Groups V*, *VI*, *VII*, and *VIII* died rather promptly of acute histamine poisoning they did not lend themselves to a similar analysis.

TISSUE EXAMINATION<sup>4</sup>

All of the animals that died of perforated ulcer with peritonitis—*Groups I, II, III, IV*—showed the familiar pathology of an acute and fulminating type of peptic ulcer of the stomach or duodenum, with no discernible gross or histological differences among specimens from the different groups.

The animals that died of acute histamine poisoning—*Groups V, VI, VII, VIII*—

<sup>4</sup> The writers are indebted to Dr. Wm. B. Hawkins, of the Department of Pathology, for confirmation of the tissue examinations.

also showed uniform changes among themselves, consisting of pulmonary hemorrhages, areas of pulmonary atelectasis and meningeal hemorrhages. There were no discernible gross or histological differences among specimens from the different groups. The pathology seen in these animals also did not differ from that seen in control animals not pre-treated with thyroid substance which were killed by an overdose of histamine for purposes of comparison. The animals treated with thiouracil showed the characteristic goitrous changes.

#### DISCUSSION

Thyroidectomy obviously accelerates the rate of development of histamine-induced peptic ulcer. The data presented do not permit the conclusion that the perforated ulcers were caused only by excess gastric acid secretion but it seems likely that

TABLE I. EFFECT OF THYROID ACTIVITY ON HISTAMINE-INDUCED PEPTIC ULCER

GROUP	PRE-HISTAMINE TREATMENT	BMR, CAL/HR/ KG $\frac{1}{4}$	MEAN SURVIVAL TIME, HRS.	CAUSE OF DEATH	REMARKS
I (9) <sup>1</sup>	None	3.38	161 ± 35 <sup>2</sup>	Perforated peptic ulcer with peritonitis	2 survivors, histamine-resistant, no ulcer <sup>3</sup>
II (8)	Benadryl		123 ± 35	Perforated peptic ulcer with peritonitis	1 survivor, histamine-resistant, no ulcer <sup>3</sup>
III (11)	Thyroidectomy	2.94	56 ± 8	Perforated peptic ulcer with peritonitis	1 survivor, histamine-resistant, no ulcer <sup>3</sup>
IV (11)	Thiouracil	2.85	125 ± 29	Perforated peptic ulcer with peritonitis	1 survivor, histamine-resistant, no ulcer <sup>3</sup>

<sup>1</sup> Number of animals. <sup>2</sup> Standard Error.

<sup>3</sup> Histamine-resistant animals were not included in calculations of mean survival times.

it was an important factor. The failure of benadryl to influence the course of ulcer induction confirms the work of other workers (12).

A sharp contrast in survival times is demonstrated between the thyroidectomized animals and those pre-treated with thiouracil. In both groups the deprivation of thyroxine or its inactivation was reflected in the fall of metabolic rate, gain in weight, decreased physical activity and growth of coat. These results suggest that the thyroid gland in addition to its role of energy metabolism regulator, possesses a function which is related to gastric secretion and possibly to resistance of the gastric mucosa to erosion. The data at hand do not permit a decision as to whether this function depends upon the anatomical integrity of the gland or upon its production of a specific substance other than thyroxine. It is interesting that thyroxine induces an increased secretion of intestinal juice which persists long after the effect on energy metabolism has subsided (13). From a consideration of the data now available it appears as if the thyroid gland exercises some sort of control over the 'acid-base balance' of the gastrointestinal tract.

No other account has been found of histamine sensitivity as a manifestation of the hyperthyroid state. One can only speculate on its relationship to the frequency of pneumonia and to the skin sensitivity seen in hyperthyroid subjects.

#### SUMMARY

The frequency, time of development, course, outcome and pathology of histamine-induced peptic ulcer in the guinea pig is unaffected by the administration of benadryl. Removal of the thyroid gland, with preservation of the parathyroids, reduces significantly the time required for perforation of histamine-induced peptic ulcer. Thiouracil in amounts adequate to produce depression of the B.M.R. and characteristic goitrous changes does not produce any significant variation from normal in frequency, development, course, outcome or pathology of histamine-induced peptic ulcer. The difference between this group and the thyroidectomized group is quite distinct. Guinea pigs made hyperthyroid by the administration of desiccated thyroid by mouth or crystalline thyroxine parenterally in amounts adequate to cause a rise in metabolic rate, loss of weight, hyperactivity and loss of hair, show a striking hypersensitivity to acute histamine poisoning. On this account it was impossible to investigate the effect of the hyperthyroid state on the formation, course or outcome of histamine-induced peptic ulcer.

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# EFFECT OF DIET ON LIVER REGENERATION IN PARTIALLY HEPATECTOMIZED RATS

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**I**N A recent article Denton and Ivy (1) reported that a liver diet enhanced liver regeneration in partially hepatectomized rats. These investigators found that liver restoration was faster in a liver-fed series of rats than in a control series on stock diet (Purina Chow). The present study was undertaken to determine whether the increased rate of regeneration in Ivy and Denton's investigation was primarily due to the high protein content of liver or to a specific substance in the liver.

## METHODS

White male albino rats weighing from 225 to 325 gm. were used. All the rats were pre-fed a specific diet for 3 days prior to surgery. On the fourth day the animals were weighed and surgery was performed. With the aid of ether anesthesia the anterior abdominal wall was shaved and a mid-line incision was made. The left lateral and median lobes of the liver were isolated and freed from their ligamentous attachments to the stomach and diaphragm. A small hemostat was placed upon the branches of the hepatic vein and artery supplying these two lobes. The pedicle of these lobes was ligated and transected and the abdominal wall closed with two layers of black silk. The removed segment of liver was weighed moist after surgery and then placed in an oven at 100°C. The liver was desiccated for a period of 3 days or until a constant dry weight was obtained. Following surgery all animals were again placed on the same diet they were fed prior to surgery. Feeding was *ad libitum*. On the 11th day post-operatively all animals were killed. The body weight was recorded, the regenerated liver removed, weighed moist and then placed into the oven to be desiccated.

The 5 diets used were as follows:

*Diet 1.* 60 per cent casein synthetic diet.

*Diet 2.* 60 per cent powdered whole liver diet. The whole powdered liver used in this diet was manufactured by VioBin Corporation, Monticello, Illinois. This product is a whole, defatted dry hog liver, processed at 37°C.

*Diet 3.* 60 per cent casein diet with Lilly's 343 Liver Extract. The amount of '343' to be added to the balanced diet was calculated from the average amount of coagulated liver consumed by a 250 to 300-gm. rat each day. This was about 11 gm/day. Each gm. of raw hog's liver yields 0.0425 gm. of '343' liver extract. Therefore, in our diet for every 10 gm. of the 60 per cent casein diet 0.425 gm. of liver extract was added.

*Diet 4.* Coagulated beef heart. Minced fresh beef heart boiled in a small quantity of water for 2 to 3 minutes.

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Received for publication February 10, 1949.

*Diet 5.* Coagulated liver diet. This consisted of fresh coagulated hog's liver. The liver was diced and submerged in boiling water for a period of 2 or 3 minutes. We found it necessary to use coagulated liver because fresh liver when allowed to stand in the animal cages for a period of 5 to 6 hours putrefied at such a fast rate that the animal refused to eat it after a short period.

Our synthetic diet (*Diets 1, 2 and 3*) included the following substances: dextrose, 17 per cent; cod liver oil, 3 per cent; brewer's powdered yeast, 8 per cent; U. S. P. salt mixture, 5 per cent; commercial lard, 6 per cent; and protein (casein or powdered liver) 60 per cent.

In computing the data, rats that lost over 10 per cent of their total body weight during the 11-day period from the time of surgery to time of death were not included in the statistical analysis.

The rate of liver regeneration has been computed in three different ways: 1) the ratio of liver weight to body weight at necropsy; 2) absolute increment in liver weight during the 11-day postoperative period; and 3) increment in liver weight relative to the amount of liver left in at the time of surgery. All computations were made on the basis of dry liver weight. The moisture content of the liver showed no significant variation in any of the groups studied.

The weight of the liver left in at the time of surgery was calculated by multiplying the liver weight removed (dried) by the factor 0.46. This factor is derived from the fact that on the average the left lateral and median lobes comprise 68.5 per cent of the total liver mass. The accuracy of this percentage has been checked by several investigators including Higgins and Anderson (2) and Denton and Ivy (1). Thus, if 68.5 per cent of the liver (left lateral and median lobes) is removed, 31.5 per cent of liver mass remains in the living animal, giving  $\frac{31.5\%}{68.5\%} = 0.46$  (factor). From the liver weight at the time of death is subtracted the liver weight left in at the time of surgery to give the value called the absolute increment.

Relative increment is calculated by dividing the increase in liver weight between the time of partial hepatectomy and the time of death (11 days later) by the liver left in at the time of surgery. Thus, the relative increment is equal to the number of gm. of liver regenerated per gm. of liver left in at the time of surgery.

#### RESULTS AND DISCUSSION

The results are summarized in table 1. It will be noted that the coefficients of variation in each group are smaller for liver weight/body weight ratio (LW/BW) than for the absolute or relative increment. The absolute increment does not take body weight into account. The relative increment takes body weight into account indirectly by relating the increment to the weight of the liver left at the time of hepatectomy. However, we have calculated for all 5 groups the correlation coefficients for the relation of weight of liver left at time of hepatectomy to magnitude of increment and found no evidence of a correlation between these values (the correlation coefficients were: +0.11, +0.15, -0.29, +0.32, -0.43). The validity of the use of the liver weight/body weight ratio is supported by our failure to find a significant correlation between this ratio and the body weight itself, indicating that the value of the ratio is independent of the body weight within the range of weights

studied here. The correlation coefficients of the relation of body weight to the ratio LW/BW for the 5 groups were:  $-0.19$ ,  $-0.60$ ,  $-0.55$ ,  $-0.82$ , and  $-0.25$ .

TABLE I. RATE OF LIVER REGENERATION IN RATS ON VARIOUS DIETS

GROUP NO. AND DIET	NO. OF RATS	BODY WT. AT TIME OF HEPATECTOMY	ABSOLUTE INCREMENT IN DRY WT. OF LIVER	RELATIVE INCREMENT IN DRY WT. OF LIVER	LW/BW RATIO AT NECROPSY ( $\times 10^4$ )
1. 60% casein	9	$273 \pm 6.8$ $C = 7.0\%$	$1.74 \pm 0.8$ $C = 12.6\%$	$1.91 \pm 1.0$ $C = 15.2\%$	$101 \pm 3.0$ $C = 8.4\%$
2. 60% powdered liver	16	$288 \pm 6.0$ $C = 8.3\%$	$2.21 \pm 0.08$ $C = 14.0\%$	$2.59 \pm 0.10$ $C = 16.2\%$	$110 \pm 2.9$ $C = 10.0\%$
Difference between groups 1 and 2		+15 $t = 1.7$	+0.47 $t = 4.1^{**}$	+0.68 $t = 4.6^{**}$	+9 $t = 2.16^*$
3. 60% casein with liver extract	9	$292 \pm 8.3$ $C = 8.6\%$	$1.37 \pm 0.06$ $C = 14.6\%$	$1.71 \pm 0.06$ $C = 11.7\%$	$81 \pm 2.5$ $C = 8.6\%$
Difference between groups 1 and 3		+19 $t = 1.8$	-0.37 $t = 3.7^{**}$	-0.20 $t = 1.7$	-20 $t = 5.2^{**}$
4. Whole beef heart	6	$314 \pm 2.9$ $C = 2.2\%$	$1.71 \pm 0.06$ $C = 8.8\%$	$1.71 \pm 0.12$ $C = 18.1\%$	$89 \pm 2.4$ $C = 6.6\%$
Difference between groups 1 and 4		+41 $t = 5.3^{**}$	-0.03 $t = 0.3$	-0.20 $t = 1.3$	-12 $t = 3.0^{**}$
5. Whole liver	13	$293 \pm 7.0$ $C = 8.2\%$	$2.15 \pm 0.07$ $C = 11.2\%$	$2.28 \pm 0.10$ $C = 16.7\%$	$112 \pm 2.2$ $C = 7.3\%$
Difference between groups 4 and 5		+21 $t = 2.1^*$	+0.44 $t = 4.0^{**}$	+0.057 $t = 3.2^{**}$	+23 $t = 6.2^{**}$

The values in this table are mean values with their respective standard errors.  $C$  = coefficient of variation ( $C = \frac{s}{\bar{x}} \times 100$ ) where  $s$  is the standard deviation of the distribution and  $\bar{x}$  is the

mean;  $t$  values have been calculated from the formula  $t = \bar{x} \sqrt{\frac{n_1 n_2 (n_1 + n_2 - 2)}{(n_1 + n_2) S^2}}$  where  $\bar{x}$  is the difference between the two means being compared,  $n_1$  is the number of items in the first group and  $n_2$  the number in the second group and  $S^2$  is the pooled sum of the squares of deviations of the items from their respective means.

A single asterisk after a  $t$  value indicates that the value is statistically significant at the 5% level of probability, a double asterisk at the 1% level and no asterisk indicates that a difference as great or greater than the one observed would be expected to occur by chance due to random sampling error oftener than 5 times in 100 similar experiments.

While these values as a group do not indicate a significant correlation, the fact that they are all negative is suggestive and it is probable that if a wider range of weights was examined a significant negative correlation would result. This would suggest that the rats with lower body weight tend to regenerate liver faster.

Inasmuch as the differences which are of greatest interest are statistically significant by all the criteria for measuring regeneration, the relation of a single criterion is not critical in this study. However, the above analysis suggests that LW/BW may be useful for future studies, perhaps with a correction factor for body weight.

The results presented in table 1 confirm the findings of Denton and Ivy in regard to the enhancing action of whole fresh liver diet on liver regeneration. In addition they reveal that this same beneficial effect is exerted by powdered whole liver incorporated into a synthetic diet. In each case, a comparison with another diet of equally high protein content (whole beef heart or synthetic diet with casein) shows that the effect cannot be attributed to the high protein content of the diet. Denton and Ivy found crude liver extract parenterally to be ineffective in promoting liver regeneration. Our studies show that crude liver extract is ineffective also when given orally. In fact, it tended to inhibit regeneration. As in the studies of Denton and Ivy (1), no gross or microscopic evidence of fattiness of the liver was present with any of these diets.

#### SUMMARY

In partially hepatectomized rats on a balanced synthetic diet, liver regeneration was more rapid in the animals fed a diet containing powdered whole liver as the source of protein than in those on casein. Similarly, freshly cooked whole pig's liver was superior to freshly cooked whole beef heart in supporting liver regeneration. A supplement of crude liver extract in the diet failed to enhance liver regeneration.

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# CONTROL OF LIVER REGENERATION AND NUCLEIC ACID CONTENT BY THE THYROID, WITH OBSERVATIONS ON THE EFFECTS OF PYRIMIDINES<sup>1</sup>

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IT WAS stated by Higgins (1) that feeding desiccated thyroid enhances the regeneration of livers in rats after removal of 75 per cent of the organ. The administration of thyroid was not begun until the day of operation, and no effects were noted until 7 to 14 days afterward, the liver being 20 per cent larger than in the control group at the end of 4 weeks. As Fogelman and Ivy (2) point out, however, liver regeneration is normally practically complete before the observed differences occur, hence it is doubtful if this can be called an effect on regeneration. It is, of course, true that an effect on liver size was demonstrated, and this is confirmed by the fact that unoperated animals under thyroid medication also showed liver hypertrophy. A similar effect induced by thyroxin injection upon the size of normal liver was observed by Sternheimer (3). The opposite condition—hypothyroidism—was investigated by Drabkin (4) who found that in the thyroidectomized rat liver regeneration, 14 days after lobectomy, was of smaller magnitude than normal, but "was not strikingly interfered with."

The results which we are here reporting show that thyroid medication significantly enhances, and that (at least in young animals) thyroidectomy significantly depresses liver regeneration. In view of the goitrogenic action of certain thiopyrimidines the effect of administering these was also investigated, another purpose in doing so being the relation of pyrimidines to the nucleic acids, and the possible competitive inhibition of these by the thiopyrimidines. It was found that pyrimidines have no effect, but that thiopyrimidines are capable of depressing regeneration; and that the latter effect is not due to suppression of thyroid hormone formation.

## METHODS

Inbred rats of the Wistar strain, varying in age from 2 to 6 months, according to the nature of the experiment, as indicated later, were used. Sex appeared to have no influence on the results. Partial hepatectomy was performed by removing the left lateral and median lobes. It was first noted by Higgins and Anderson (5) in 1931, and amply confirmed, that this removes with sufficient constancy about two-thirds of the liver. The amount of regeneration can be variously calculated; we have used the following formula: % regeneration =  $\frac{\text{wt. of liver at autopsy} - \frac{1}{3} \text{amount removed}}{\text{amount removed}}$

$\times 100$ .

Received for publication February 3, 1949.

<sup>1</sup>A preliminary report of this work was made at the Meeting of the American Physiological Society, Minneapolis, Sept. 1, 1948.

<sup>2</sup>Aided by a grant from the Division of Research Grants and Fellowships of the National Institute of Health, U. S. Public Health Service.

This represents, in percentage, approximately the amount of removed liver replaced by new tissue.

All surviving animals were killed 96 hours after lobectomy, this being chosen because at this time the most rapid phase of regeneration has been completed. The standard diet consisted of 25 per cent casein, 50 per cent sucrose, 20 per cent Crisco, 5 per cent salt mix (Phillips and Hart, 1935); Vipenta, 0.5 ml/kg. There is evidence of a somewhat conflicting nature that diet affects liver regeneration, and it was therefore necessary to avoid this variable. The experimental animals were not 'pair fed' with their controls, but the amount eaten by each animal was noted, and the diet of the control group held down to approximately the same level. We have not included the evidence in tables, but wish to say that there was no significant statistical deviation in the amounts of food eaten by the control and experimental groups, and that the changes of weight in the animals were also of the same approximate order. We therefore are confident that the results are not affected by these particular variables.

Care was taken that any group of experimental animals should be of similar age as their controls, since animals before maturity (i.e. younger than 3-4 months) regenerate liver more rapidly than older animals. This will appear later.

Thyroid was administered in the form of the desiccated gland, mixed with the food when given in large amounts (500 mg/day), introduced directly into the back of the mouth when given in small amounts (3.5 mg/day). After some experimentation the pyrimidines and thiopyrimidines were administered as 1 per cent by weight of the diet, mixed with the food. All of these substances were given for either 10 or 20 days before hepatectomy (as hereinafter indicated) as well as during the four-day period of regeneration.

Nucleic acids were determined according to the method of Schmidt and Thannhauser (6) and expressed in terms of phosphorus.

## RESULTS

*Effects of Thyroidectomy and Thyroid Administration.* In table 1, it will be seen that in 4- to 6-month-old rats the regeneration 96 hours after lobectomy on a restricted diet is  $62 \pm 2.7$  per cent (on an *ad. lib.* diet this would in animals of this age be considerably higher). In animals thyroidectomized six weeks earlier, though slightly lower, it is not significantly different. When dried thyroid is given, however, in amounts that are sufficient to produce definite hyperthyroidism (500 mg. dried thyroid, per rat of 200-300 gm/day for 10 days), there is a significant increase in the rate of regeneration, amounting to 77 per cent at the end of the four-day period. In thyroidectomized animals given this amount of thyroid, the chemical picture was similar to that of the normal animal which had been made hyperthyroid by medication; but none of the 7 animals studied survived partial hepatectomy.

The ribonucleic acid fraction (PNA) is somewhat diminished in the pre-lobectomized liver six weeks after thyroidectomy, but is not affected in the regenerated liver; the deoxyribonucleic acid (DNA) fraction is unchanged. When the normal animal is given large amounts of thyroid, however, there is a considerable increase in the nucleic acids, especially the PNA fraction, in both the pre-lobectomized and regenerating liver.

After thyroidectomy, the water content of the regenerating liver is slightly reduced, as is the nitrogen content. On the other hand, thyroid administration, while it has no effect on the water content, significantly increases the percentage of nitrogen in both the pre-lobectomized and regenerating organ. This is in accord with the observations of Sternheimer (*loc cit.*) on the protein content of *normal* liver after thyroxin injection. On the whole, then, in this age group thyroidectomy

induces somewhat equivocal results, while after heavy dosage of thyroid, effects occur indicating increased capacity for regeneration, as well as mobilization of proteins, including nucleoproteins.

TABLE I. RELATION OF THYROID AND THIOPYRIMIDINE EFFECTS (4- TO 6-MONTH-OLD ANIMALS)

	PRE-LOBECTOMIZED LIVER						REGENERATED LIVER (96 HOURS)					
	Nucleic acids			Dry wt. Wet wt.	Total N	%	Nucleic acids			Dry wt. Wet wt.	Total N	Regen- eration
	PNA	DNA	Total				PNA	DNA	Total			
Controls (34) <sup>1</sup> ...	98	21	119	30	3.13	%	109	21	130	30	2.97	62
	±1.2	±0.4	±1.3	±0.2	±.027		±1.5	±0.1	±1.5	±0.1	±0.12	±2.7
Thyroidectomized (21, 18) ..	89	20	109	30	3.06		108	20	128	26	2.77	56
	±2.9	±2.3	±3.6	±0.2	±.084		±2.5	±1.6	±3.0	±0.4	±.061	±4.7
Thiouracil, 20 days (12, 9)...	102	20	122	30	2.90		107	19	126	26	2.67	49
	±2.3	±0.5	±2.3	±0.9	±.109		±1.9	±0.8	±2.1	±0.7	±.111	±2.6
Thyroidectomized + thiouracil, 20 days (11, 3)...	87	18	105	28	3.05		108	18	126	28	2.95	58
	±1.1	±0.27	±1.1	±0.5	±.085		±1.0	±0.4	±1.1	±0.4	±.068	±7.6
Dried thyroid, 500 mg/day, 10 days (22, 16).....	122	26	148	29	3.49		129	24	153	28	3.24	77
	±1.0	±1.9	±2.1	±0.1	±.005		±1.7	±1.4	±2.2	±0.4	±.006	±3.6
Thyroidectomized + dried thyroid, 500 mg/day, 10 days (8, 7)....	139	28	167	28	3.36	All died						
	±1.8	±0.7	±1.9	±0.3	±.113							
Thiouracil + dried thyroid, 500 mg/day 10 days (13)...	137	28	165	28	3.35		146	25	171	26	3.42	72
	±1.1	±1.1	±1.6	±0.5	±.080		±1.8	±0.9	±2.1	±0.3	±.083	±4.6
20 days (6)....	133	26	159	28	3.44	All died						
	±1.5	±2.1	±2.6	±0.3	±.065							

<sup>1</sup> Numbers in parentheses refer to number of experimental animals. Where there are two numbers in parentheses, the second refers to the number that survived partial hepatectomy for regeneration studies. Deviation measure = Standard Deviation of mean =  $\sqrt{\frac{\sum d^2}{n(n-1)}}$

These results are to be compared with experiments shown in table 2. In this case, the animals were younger, being approximately 8 to 9 weeks old at partial hepatectomy. The diet of the control group was again limited to approximately that of the thyroidectomized animals, as were the thyroid-fed. In this group the regen-

eration of the 'normal' animals was higher than in the older group of similar animals, amounting to 99 per cent at the end of 96 hours. The thyroidectomized groups, how-

TABLE 2. RELATION OF THYROID AND THIOPYRIMIDINE EFFECTS (2-MONTH-OLD ANIMALS)

	PRE-LOBECTOMIZED LIVER						REGENERATED LIVER (96 HOURS)					
	Nucleic acids			Dry wt.	Total N	%	Nucleic acids			Dry wt.	Total N	Regen-
	PNA	DNA	Total	Wet wt.	%	PNA	DNA	Total	Wet wt.	%	era-	
Controls (13) <sup>1</sup> ...	97	24	121	30	2.94		107	27	134	29	2.88	99
	±3.5	±1.8	±3.9	±0.3	±0.058		±3.9	±1.7	±4.2	±0.6	±0.085	±7.7
Thyroidect.												
Without thiouracil, 20 days (11) <sup>1</sup> .	82	19	101	29	2.80		97	21	118	25	2.60	56
	±1.5	±0.6	±1.6	±0.3	±0.077		±1.4	±0.6	±1.5	±0.4	±0.081	±2.0
With 1% thiouracil, 20 days (21, 15) <sup>1</sup> .....	96	20	116	30	2.89		126	20	146	27	2.81	61
	±2.6	±0.5	±2.7	±0.3	±0.069		±2.5	±0.5	±2.6	±0.5	±0.028	±3.3
Thyroidect. with 3.4 mg. dried thyroid daily												
Without thiouracil 10 days (12, 10) <sup>1</sup> .....	111	24	135	31	3.00		114	21	135	27	3.06	97
	±2.1	±0.8	±2.2	±2.2	±0.079		±1.6	±2.4	±2.9	±0.5	±0.211	±7.8
30 days (10, 9) <sup>1</sup> ...	113	25	138	29	2.79		130	26	156	25	2.74	82
	±2.9	±1.1	±3.1	±0.3	±0.047		±2.9	±0.7	±3.0	±0.4	±0.064	±6.9
With 1% thiouracil 10 days (13, 9) <sup>1</sup> .....	112	21	133	29	3.05		121	23	144	28	2.97	68
	±2.7	±1.0	±2.9	±0.2	±0.071		±1.4	±0.8	±1.6	±0.4	±0.133	±6.2
20 days (22, 11) <sup>1</sup> .....	112	24	136	30	3.24		129	24	153	27	2.99	66
	±1.3	±0.5	±1.4	±0.3	±0.061		±3.5	±1.1	±3.6	±0.5	±0.075	±4.7

<sup>1</sup> Numbers in parentheses refer to number of experimental animals. Where there are two numbers in parentheses, the second refers to the number that survived partial hepatectomy for regeneration studies. Deviation measure = Standard Deviation of mean =  $\sqrt{\frac{\sum d^2}{n(n-1)}}$ .

ever, whose thyroids had been removed 20 days before hepatectomy, regenerated only to the extent of 56 per cent, or no better than the older thyroidectomized ani-

mals and unmistakably less than the controls. There was also a reduction in the nucleic acids, both PNA and DNA, in both the pre-lobectomized and regenerating liver, as well as a tendency towards a decrease in the total nitrogen. The reason for diminishing the interval between thyroidectomy and hepatectomy to 20 days, instead of 6 weeks as in the group previously discussed, was to reduce the age level of the group for comparison with a similar age level in controls.

Another group of animals of this age level was, immediately after thyroidectomy, put on a replacement thyroid therapy. The intent, in this case, was not, as previously, to create a hyperthyroid animal, but simply to restore the putative amount of normal thyroid secretion. From the data of Astwood and Dempsey (8), this was computed at 3.5 mg. of desiccated thyroid per day per rat for rats of this age and weight (about 150 gm.). Under these conditions, the regenerative capacity of the liver was restored to normal in animals where hepatectomy was performed 10 days after thyroidectomy, and who received replacement thyroid medication during this period and the period of regeneration. When hepatectomy was performed under similar conditions 20 days after thyroidectomy, the regeneration was almost as great (table 2, row 4).

The nucleic acids of the controls in this series was higher after lobectomy than in the older animals. After thyroidectomy, the fall in nucleic acids, particularly PNA, was evident, though not large. On the other hand, there was a definite increase when dried thyroid was administered, though not as much as in the earlier series, on massive doses of the hormone.

*Effect of Pyrimidines.* In view of the fact that pyrimidines constitute a part of the nucleic acid molecule, it was felt that administration of pyrimidines might affect the nucleic acid mobilization and possibly show a differential effect, since uracil is found in ribonucleic acid, and thymine in deoxyribonucleic acid. Cytosine is found in both, but we did not have any of this material at our disposal. After some experimentation, based on our experience with the thiopyrimidines, both uracil and thymine (Schwartz) were given as 1 per cent of the diet for period a of 10 to 20 days. It will be seen in table 3 that in respect to the things we were observing, these substances were entirely without effect.

*Effect of Thiopyrimidines.* Two-thiouracil, 5-methyl-thiouracil (thiothymine), and 6-propylthiouracil<sup>8</sup> were given to groups of 4- to 6-month-old rats as 1 per cent of the diet. In this amount, fed over a period of 10 days before hepatectomy (as well as during regeneration), the substances caused small decreases in regeneration (table 4); when the administration was continued for 20 days before hepatectomy, the decrease became much more marked, and was, if anything, greater than in thyroidectomized animals of this age level. Nevertheless, except in the case where thiothymine is given for 20 days, there was practically no effect on the nucleic acid content. After thiothymine and propylthiouracil administration for 10 days, there was a considerable increase in the total nitrogen, apparently indicating protein mobilization, but on continuance of the medication for 20 days, the nitrogen returned to normal. The significance of this is not clear. There is no reason to doubt that

<sup>8</sup>We are indebted to Dr. E. B. Astwood for the thiouracil and to Dr. Stanton E. Hardy of the Lederle Division of the American Cyanamid Company, for the propylthiouracil used in this work.

the protein mobilization would also have been observed after 10 days of thiouracil medication, but this experiment was not performed.

*Relation of Thyroid to Thiouracil Effects.* When thiouracil medication was superimposed upon thyroidectomy (table 1), we were unable to keep more than 3 out of 11 of the older animals alive after hepatectomy. In these there was no summation of effect, the results corresponding very closely to that of thyroidectomy alone. In young animals (2 months old), with a shorter interval after thyroidectomy, the results were somewhat different. When thiouracil was given to such animals, the regeneration was reduced as in thyroidectomy (table 2, row 3), but,

TABLE 3. EFFECT OF PYRIMIDINES (4- TO 6-MONTH-OLD ANIMALS)

	PRE-LOBECTOMIZED LIVER						REGENERATED LIVER (96 HOURS)					
	Nucleic acids			Dry wt.	Total N	%	Nucleic acids			Dry wt.	Total N	Regeneration
	PNA	DNA	Total	Wet wt.	%	PNA	DNA	Total	Wet wt.	%	%	%
Controls (34) <sup>1</sup> ...	mg. P/100-gm. wet wt.						mg. P/100-gm. wet wt.					
	98	21	119	30	3.13		109	21	130	30	2.97	62
	±1.2	±0.4	±1.3	±0.2	±0.027		±1.5	±0.1	±1.5	±0.1	±0.012	±2.7
Uracil 1%												
10 days (4) <sup>1</sup> ...	93	21	114	31	3.08		106	21	127	30	3.22	64
	±0.6	±0.3	±0.7	±0.5	±0.086		±1.2	±0.0	±1.1	±1.2	±0.215	±4.8
20 days (12) <sup>1</sup> ...	95	19	114	30	3.01		105	21	126	30	2.81	62
	±3.4	±1.1	±3.5	±0.3	±0.068		±2.4	±0.9	±2.6	±0.7	±0.131	±4.7
Thymine 1%												
10 days (24)...	91	21	112	30	3.74		112	21	133	31	3.04	63
	±0.8	±0.4	±0.9	±0.2	±0.059		±3.6	±0.3	±3.6	±0.4	±0.067	±3.7
20 days (18)...	92	22	114	30	3.07		110	22	132	30	2.92	57
	±1.8	±0.4	±3.4	±0.3	±0.057		±2.4	±0.4	±2.4	±0.5	±0.055	±1.2

<sup>1</sup> Numbers in parentheses refer to number of experimental animals. Where there are two numbers in parentheses, the second refers to the number that survived partial hepatectomy for regeneration studies. Deviation measure = Standard Deviation of mean =  $\sqrt{\frac{\sum d^2}{n(n-1)}}$ .

surprisingly, there was, after partial hepatectomy, a rise in the ribonucleic acid fraction.

When, in normal animals, dried thyroid and thiouracil administration were combined the result seemed to depend on the amount of thyroid fed. With large amounts of thyroid (table 1), the result was practically as though no thiouracil had been given. This was explicable on the ground that the thyroid effect was dominant, and the conclusion was buttressed by the fact that when only replacement amounts of desiccated thyroid were given with thiouracil (table 2) the latter was able to combat the effect of the administered thyroid as to regeneration, the latter being reduced almost to the same level as that of the thyroidectomized animals. However, the typical increase of nucleic acids seen after thyroid medication was not nullified by thiouracil.

## DISCUSSION

The evidence points to a control of liver regeneration as well as of nucleic acid and protein mobilization by the thyroid. On the other hand, the fact that the effect of thyroidectomy on regeneration is clearly seen only in younger animals seems to indicate that as the animal approaches the adult state, control of regeneration can be taken over by other forces in the body. The thyroid hormone stimulates regeneration by the liver, and it may be that the greater regenerative capacity of the organ

TABLE 4. EFFECT OF THIOPYRIMIDINES (4- TO 6-MONTH-OLD ANIMALS)

	PRE-LOBECTOMIZED LIVER						REGENERATED LIVER (96 HOURS)					
	Nucleic acids			Dry wt. Wet wt.	Total N	Nucleic acids			Dry wt. Wet wt.	Total N	Regen- eration	
	PNA	DNA	Total			PNA	DNA	Total				
Controls (34) <sup>1</sup> ...	98	21	119	30	3.13	108	21	130	30	2.97	62	
	±1.2	±0.4	±1.3	±0.2	±0.027	±1.5	±0.1	±1.5	±0.1	±0.12	±2.7	
Thiouracil 1%, 20 days (12, 9).....	102	20	122	30	2.90	107	19	126	26	2.67	49	
	±2.3	±0.5	±2.3	±0.9	±1.00	±1.9	±0.8	±2.1	±0.7	±1.11	±2.6	
Propylthiouracil 10 days (6, 5) ..	105	21	126	29	3.55	114	22	136	27	3.47	53	
	±3.0	±0.0	±3.0	±0.4	±0.57	±1.8	±0.1	±1.8	±1.2	±1.16	±3.4	
20 days (15, 11).....	96	21	117	30	3.31	112	20	132	27	2.87	42	
	±1.5	±0.7	±1.7	±0.3	±0.39	±1.2	±0.4	±1.3	±0.4	±0.88	±3.1	
Thiothymine 1% 10 days (6, 5) ..	98	21	119	30	3.45	107	21	128	30	3.34	58	
	±2.4	±0.6	±2.5	±0.5	±1.13	±2.8	±0.3	±2.8	±1.3	±0.67	±1.3	
20 days (24, 22).....	87	17	104	30	3.06	106	18	124	30	3.00	45	
	±1.1	±0.6	±1.3	±0.2	±0.78	±2.1	±0.6	±2.2	±0.4	±0.71	±2.9	

<sup>1</sup> Numbers in parentheses refer to number of experimental animals. Where there are two numbers in parentheses, the second refers to the number that survived partial hepatectomy for regeneration studies. Deviation measure = Standard Deviation of mean =  $\sqrt{\frac{\sum d^2}{n(n-1)}}$ .

in the younger animal is a function of greater thyroid activity. The sharp drop following thyroidectomy in such animals, to the level in the older thyroidecomized animals, seems to bear this out; and the fact that it goes no lower indicates that as in the adult, other controlling factors are at work, though not in this case compensatory. The thyroid hormone also stimulates mobilization of liver nucleic acids, especially ribonucleic acid, though desoxyribonucleic acid, whose concentration is notoriously difficult to change, is also affected to some extent. According to Novikoff and Potter (8), the ribonucleic acid content is greatest during the period of most active regenera-

tion. This would be in harmony with the thyroid effect on the latter. On the other hand, on the basis of effects we have noted in other investigations, it seems to us that the necessary association of active growth with nucleic acid mobilization must be taken with some reserve. We therefore record the facts in this case without prejudice or postulate. We must also record the apparent mobilization of total protein with the same reservation.

As stated earlier in this paper, we were impelled to test the effect of pyrimidines by two major considerations. In the first place, the thiopyrimidines are goitrogenic, and it is therefore reasonable to expect that they may abolish the thyroid hormone effect on liver by preventing its synthesis. In the second place, ingested pyrimidines, despite certain evidence to the contrary (cf. Plentl and Schoenheimer (9)), might influence, in a rapidly growing organ, the nucleic acid content, and if the latter were a limiting factor, the actual growth of that organ. Also, in this connection, it is possible that the thiopyrimidines might act either as sources of pyrimidines or as competitive inhibitors of any effects the latter might have.

The results that we obtained indicate that the ingestion of pyrimidines added to a synthetic diet containing none, does not affect the synthesis of liver nucleic acid or regeneration of the organ. This tends to corroborate, as far as it goes, the evidence of Plentl and Schoenheimer.

As for the thiopyrimidines, in the amount given, they inhibited liver regeneration. This is, of course, a large dose, much above the minimum goitrogenic effect, though according to Astwood (10) not incompatible with continued existence with symptoms referable only to hypothyroidism, even when continued over a long period of time. Fogelman and Ivy (*loc cit.*) have shown that when rats were given intraperitoneal injections of 8 mg. thiouracil per 100 gm. of body weight daily following partial hepatectomy, the rate of regeneration was increased. This might correspond roughly to the ingestion of 0.1 per cent in the diet, or much less than the amount we gave. In view of these and our own findings, therefore, it appears that the effect of thiouracil, at least, is a function of the amount given. The inhibition of regeneration seen in our experiments is not a function of the anorexia and concomitant failure to gain, or actual loss of weight of the animals, since the controls were arbitrarily subjected to the same conditions. Nor is it a function of the depression of thyroid hormone synthesis, because when the normal thyroid hormone secretion was replaced by ingestion in a thyroidectomized animal, and the regeneration restored to normal, the latter was still depressed by thiouracil. We are led to believe that the thiouracil effect, whether depressant as in our case, or stimulating, as in the case of Fogelman and Ivy, is at least in part an effect either directly on the liver itself or on some other control than that furnished by the thyroid.

We set out to determine, among other things, whether thiopyrimidines would antagonize, perhaps by competitive inhibition, a possible pyrimidine effect. As we have already stated, we were unable to demonstrate this inhibition because administered pyrimidines were without effect. It is, however, not ruled out, for while it may be that ingested pyrimidines are not incorporated into nucleic acids, the pyrimidines of the latter in such case may be considered as being synthesized from other fragments of metabolism, and if so, the postulated inhibition may still occur after

such synthesis has taken place. Admittedly, we have no evidence for or against this.

#### SUMMARY

Observations have been made concerning the influence of the thyroid, and of pyrimidine and thiopyrimidine administration, upon rat liver regeneration and nucleic acid and nitrogen content. The evidence shows that the thyroid is a regulator of liver regeneration, as well as of liver nucleic acid and protein mobilization. In animals over four months old, but not in younger animals, the control over regeneration can, in the absence of the thyroid, be taken over by something else. Ingested pyrimidines have no effect on liver regeneration, or on nucleic acid and nitrogen content. Ingested thiopyrimidines, in high concentration, reduce the regenerative capacity of the liver; and this effect is not due to inhibition of the formation of thyroid hormone. A postulated inhibition of pyrimidines by thiopyrimidines was not proved; neither was it ruled out.

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# BRAIN AND PLASMA CATIONS AND EXPERIMENTAL SEIZURES IN NORMAL AND DESOXYCORTICOSTERONE-TREATED RATS<sup>1</sup>

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**A**LTHOUGH clinical studies on the efficacy of desoxycorticosterone therapy in epilepsy have yielded contradictory results (1, 2), in a few cases a significant decrease in the incidence of grand mal seizures has been noted (2). This improvement in the clinical status of the patients was apparently due to a retention of Na in the body, and a loss of K (3). Spiegel (4) administered desoxycorticosterone to rats but failed to observe a change in the convulsive threshold to electrical stimulation. On the other hand, a growing body of evidence indicates that the brain excitability of rats may be altered in a predictable manner by changes in the electrolyte balance of the body. Swinyard (5) has found a positive correlation between extracellular sodium concentration and electroshock seizure threshold in experimentally hydrated rats, and recent work in this laboratory (6) has demonstrated a lowered electroshock seizure threshold associated with low plasma Na levels in adrenalectomized rats. Evidence has also been presented which indicates that the refractory period following a major convulsion may be correlated with elevated plasma Na (7). In view of these observations it seemed desirable to reexamine the effect of desoxycorticosterone on brain excitability and to investigate the effects of excess Na, K, Ca and Mg.

## METHODS

Adult Sprague-Dawley male rats were used as experimental animals. Electroshock seizure thresholds (6, 8) and maximal seizure patterns (9) were determined by the standard methods of this laboratory.

Isotonic solutions of the salts used were injected intraperitoneally in the dosages indicated. After 1 hour, an interval found to be sufficient for the absorption of most of the fluid, the electroshock seizure thresholds were determined. The thresholds observed, expressed in milliamperes (mA), were compared with control observations made 6 hours earlier. Maximal seizure patterns were also investigated before and 1 hour after the injection of the salt solutions. They were checked at 30-minute intervals thereafter until the greatest change from normal was found.

Desoxycorticosterone acetate<sup>2</sup> (DCA) was administered by the subcutaneous implantation of six 15-mg. pellets per rat. The difference in weight of the pellets before implantation and after removal indicated that the mean amount absorbed was 1.6 mg/rat/day.

Samples of plasma and of brain were collected 23 to 31 days after the implantation of DCA pellets or 1 hour after the injection of salt solutions. They were prepared and analyzed for Na, K,

Received for publication February 14, 1949.

<sup>1</sup> This investigation was supported by a research grant from the Division of Research Grants and Fellowships of the National Institute of Health, U. S. Public Health Service.

<sup>2</sup> The desoxycorticosterone acetate was kindly supplied by Dr. Ernst Oppenheimer of Ciba Pharmaceutical Co.

Ca, Cl and water by the methods in use in this laboratory (6). Plasma samples were analyzed for Mg by the method of Kunkel, Pearson and Schweigert (10).

### RESULTS

The effects on maximal seizure pattern produced by the intraperitoneal injection of NaCl, KCl, MgCl<sub>2</sub> and CaCl<sub>2</sub> solutions and of modified Ringer's solution (*pH* 7.4) are illustrated in figure 1. Each group of 3 columns shows the duration of the phases of maximal seizures in a single animal and is typical of the responses of 10 to 25 animals treated similarly. The first column shows the control pattern, the second shows the pattern at the peak action of the injected salt, and the third column indicates the recovery of the control pattern. The injection of Ringer's solution had no effect on the seizure pattern. The injection of either NaCl or KCl solution resulted in abolition of the tonic phase and prolongation of the clonic phase, whereas

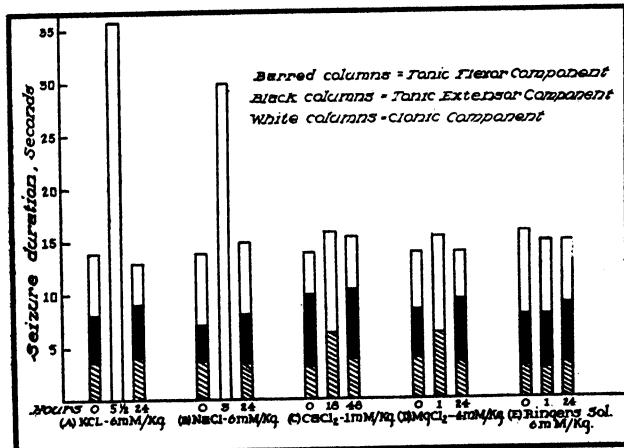


Fig. 1. EFFECTS OF INJECTED SALT SOLUTIONS on the maximal seizure patterns of rats.

the injection of CaCl<sub>2</sub> or MgCl<sub>2</sub> solution caused abolition of the tonic extensor component without change in the total duration of the seizure.

Table 1 presents the effects of the same salt solutions and of phosphate buffer on the electroshock seizure thresholds of normal rats. The injection of Ringer's solution again had no effect. Treatment with NaCl solution caused a 14 per cent rise in threshold, and CaCl<sub>2</sub> solution caused a smaller but significant rise. The injection of KCl, MgCl<sub>2</sub> or phosphate buffer resulted in a significant lowering of the threshold.

A summary of the observations made on the effect of DCA on the electroshock seizure thresholds of rats is presented in figure 2. The thresholds of 22 control and 22 treated animals were followed over a period of 31 days. The initial threshold of both groups was 20.5 mA. The controls showed no consistent change in threshold and on the final day had a threshold identical with that on the first day. The rats implanted with DCA pellets exhibited a progressive rise in threshold. This amounted

to 14 per cent of the initial value in the first 2 weeks and an additional 5 per cent in the following 17 days. The threshold at the end of 31 days was thus nearly 20 per cent above the initial level and that of the control rats.

TABLE 1. EFFECTS OF INTRAPERITONEALLY INJECTED SALT SOLUTIONS ON ELECTROSHOCK SEIZURE THRESHOLDS (EST) OF NORMAL RATS

SALT	NO. OF RATS	DOSE, mm./kg.	MEAN EST IN MA.		CHANGE IN EST MA	P
			Control	60 min. after injection		
Ringer's solution	16	6	24.5	24.8	+0.3 ± 0.2	>0.1
NaCl	16	6	24.5	27.9	+3.4 ± 0.3	<0.001
CaCl <sub>2</sub>	26	1	25.0	26.0	+1.0 ± 0.2	<0.001
KCl	24	6	27.7	23.5	-4.2 ± 0.3	<0.001
MgCl <sub>2</sub>	22	4	26.7	22.9	-3.9 ± 0.1	<0.001
Na <sub>2</sub> HPO <sub>4</sub> —NaH <sub>2</sub> PO <sub>4</sub> pH 7.4	5	2	25.1	22.9	-2.2 ± 0.2	<0.001
Na <sub>2</sub> HPO <sub>4</sub> —NaH <sub>2</sub> PO <sub>4</sub> pH 7.4	11	4	25.6	23.1	-2.5 ± 0.2	<0.001

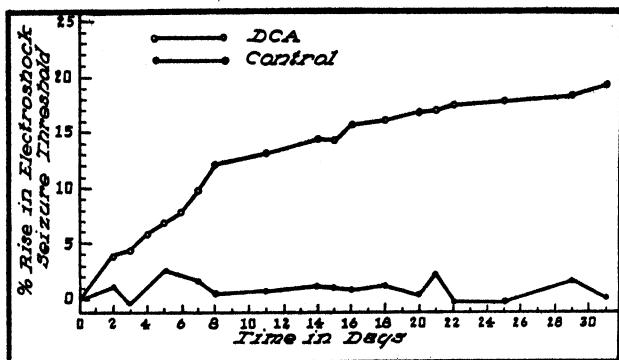


Fig. 2. EFFECTS OF DCA ON THE ELECTROSHOCK SEIZURE THRESHOLDS OF RATS.

TABLE 2. WATER AND ELECTROLYTE CONCENTRATIONS IN BRAIN CORTEX OF NORMAL AND DCA-TREATED RATS

N	PER KILOGRAM WET BRAIN			
	H <sub>2</sub> O		Na.	
	gm	mEq.	mEq.	mEq.
Normal rats	10	794 ± 1	50.0 ± 0.2	101.6 ± 0.7
DCA, 23–31 days	6	793 ± 2 (0.7)	49.1 ± 0.6 (0.4)	102.2 ± 0.4 (0.6)

N = number of pooled samples. Each pool consisted of the brain cortex from 4 rats. Figures in parentheses are P values of differences from controls.

Table 2 shows the observed concentrations of water and electrolytes in the brain cortices of normal and DCA-treated rats. At a time when the electroshock seizure thresholds of the treated animals were very significantly elevated (23–31

days after the implantation of pellets) the concentrations of water, Na, K and Cl in the brains of these animals were the same as those of normal rat brains. Single samples, each representing the pooled brains of 4 rats injected with NaCl, KCl, CaCl<sub>2</sub> or MgCl<sub>2</sub> solutions, were also analyzed and were found to contain normal amounts of Na, K, Cl and water.

Blood was collected from the rats showing the electroshock seizure threshold changes of table 1 or from strictly comparable animals. The results of the analyses of the plasma samples are shown in table 3. Only in the case of CaCl<sub>2</sub>-injected rats was there a significant change in plasma water content. In the DCA-treated animals, the plasma Na was elevated while the K and Mg were depressed to a significant extent. The intraperitoneal injection of NaCl solution caused a rise in plasma Na and a small but probably significant rise in Ca, with no change in plasma K or Mg concentrations. The plasma Ca rose after the injection of CaCl<sub>2</sub> solution but there was no change in the concentrations of the other three cations. After the administration of KCl solution there was a fall in plasma Na, a rise in plasma K, and, surprisingly, a rise in plasma Ca concentration. The injection of MgCl<sub>2</sub> solution resulted in a rise in the plasma Mg concentration accompanied by a fall in both Na and K concentrations.

#### DISCUSSION

With respect to their effects on maximal seizure patterns, the 4 salts, NaCl, KCl, CaCl<sub>2</sub> and MgCl<sub>2</sub>, fall into 2 groups. The chlorides of the 2 monovalent cations, which abolish the tonic phase of the convulsion, appear to share some of the properties of anticonvulsant drugs (11). The chlorides of the 2 bivalent cations, however, abolish only the tonic extensor component and do not affect the total duration of the convulsion. Since NaCl and CaCl<sub>2</sub> raise the electroshock seizure threshold while KCl and MgCl<sub>2</sub> lower it, it is apparent that the effect of an intraperitoneally injected salt solution on the maximal seizure pattern is not correlated with its effect on the electroshock seizure threshold.

Earlier work done in this laboratory showed that the rat brain has a remarkable ability to preserve a normal content of Na and K under conditions of low plasma Na and markedly elevated plasma K (6). The work presented here indicates that the brain can likewise maintain normal concentrations of Na and K during treatment with DCA, when plasma Na is chronically elevated and plasma K decreased. This observation is in agreement with that of Hoagland and Stone (12) who, using similar analytical methods, found no change in brain Na and K following treatment of rats with DCA. A discussion of the implications of this constancy of brain Na and K concentrations is beyond the scope of this paper.

Clearly, the decreased brain excitability of DCA-treated rats, indicated by the rise in electroshock seizure threshold, is not the result of changes in brain water, Cl, Na or K concentrations. The same is undoubtedly true of the alterations in brain excitability produced by intraperitoneal injection of the 4 salt solutions. This is indicated by the fact that analyses of single samples of brain from the injected rats revealed no differences from normal. The explanation of the changes in brain excitability must lie elsewhere.

TABLE 3. WATER AND CATION CONCENTRATIONS PER KILOGRAM WET PLASMA IN RATS TREATED WITH SALT SOLUTIONS OR DCA

TREATMENT	HO	Na		K	Ca	$\text{mEq.}$	N	$\text{mEq.}$	N	$\text{Mg}$
		$\text{mEq.}$	N							
No treatment		921 $\pm$ 2	13	142.5 $\pm$ 0.5	23	4.70 $\pm$ 0.07	23	5.50 $\pm$ 0.09	13	2.34 $\pm$ 0.04
DCA, 23-31 days		922 $\pm$ 1	14	152.7 $\pm$ 0.9 (0.7)	14	3.35 $\pm$ 0.23 (0.001)	14	5.52 $\pm$ 0.05 (0.6)	8	1.78 $\pm$ 0.07 (0.001)
NaCl, 1 hr.		924 $\pm$ 1	10	146.5 $\pm$ 1.0 (0.2)	10	4.52 $\pm$ 0.17 (0.7)	10	5.85 $\pm$ 0.06 (0.02)	8	2.30 $\pm$ 0.04 (0.001)
CaCl <sub>2</sub> , 1 hr.		931 $\pm$ 1	10	141.8 $\pm$ 0.5 (0.3)	10	4.36 $\pm$ 0.16 (0.1)	10	7.53 $\pm$ 0.11 (0.001)	12	2.33 $\pm$ 0.03 (0.9)
KCl, 1 hr.		920 $\pm$ 2	10	135.4 $\pm$ 0.8 (0.5)	10	8.64 $\pm$ 0.09 (0.001)	10	6.22 $\pm$ 0.13 (0.001)	8	2.45 $\pm$ 0.05 (0.1)
MgCl <sub>2</sub> , 1 hr.		923 $\pm$ 1	10	132.2 $\pm$ 0.9 (0.7)	10	3.31 $\pm$ 0.08 (0.001)	10	5.52 $\pm$ 0.04 (0.9)	9	12.12 $\pm$ 0.19 (0.001)

N = number of samples; 4-7% of the samples consisted of the plasma from individual rats, the remainder were the pooled plasmas of 2 to 6 rats.  
 Figures in parentheses are P values of differences from controls.

It has been shown that changes in the degree of hydration produce corresponding changes in the electroshock seizure threshold of the rat (3). However, the changes in electroshock seizure threshold resulting from DCA treatment or the injection of salt solutions cannot be explained on this basis. Only the injection of  $\text{CaCl}_2$  solution, which raised the threshold, caused a significant difference in plasma water content, and in this case the increased water content would tend to lower, rather than raise, the electroshock seizure threshold. It has already been shown (6) that no correlation can be made between plasma K concentration and electroshock seizure threshold. This is further emphasized by the fact that the plasma K was significantly lowered in both DCA-treated and  $\text{MgCl}_2$ -treated rats. Since in the experiments reported here alterations in plasma Mg concentration were always accompanied by inverse changes in plasma Na, it is impossible to determine whether the plasma Mg concentration exerts any influence on brain excitability. Changes in plasma Ca concentration, if not masked by changes in plasma Na, may affect brain excitability. Of the factors tested, only the increased plasma Ca could account for the elevated electroshock seizure threshold of the  $\text{CaCl}_2$ -injected rats. It is also probable that the reduction in electroshock seizure threshold produced by the injection of phosphate buffer was due to a lowering of plasma Ca concentration. Although changes in electroshock seizure threshold can be produced without alterations in plasma Na, it is apparent that in all situations examined here and in adrenalectomized rats (6) any variation of more than 3 mEq/l in plasma Na concentration is accompanied by a change in electroshock threshold. The plasma Na concentration thus appears to be an important factor in determining brain excitability.

#### SUMMARY

Normal rats treated with DCA or injected with isotonic  $\text{NaCl}$  or  $\text{CaCl}_2$  solution have elevated electroshock seizure thresholds. Rats injected with isotonic  $\text{KCl}$  or  $\text{MgCl}_2$  solution or phosphate buffer show lowered thresholds for electroshock convulsions. The injection of Ringer's solution produces no change in electroshock seizure threshold. Alterations in maximal seizure pattern produced by injection of the salt solutions cannot be correlated with the effects on electroshock seizure threshold. The brains of rats treated for 23 to 31 days with DCA contain normal concentrations of water, Na, K and Cl. This is apparently also true of the brains of rats injected with  $\text{NaCl}$ ,  $\text{KCl}$ ,  $\text{CaCl}_2$  or  $\text{MgCl}_2$  solutions. The changes in brain excitability produced by treatment with DCA or the 4 salt solutions can be correlated with changes in plasma Na or Ca concentrations. An increase in plasma Ca, if not masked by a change in plasma Na, is accompanied by an elevation of the electroshock threshold. Any change of more than 3 mEq/l in plasma Na is accompanied by a change of electroshock seizure threshold in the same direction.

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# EFFECTS OF ADRENALECTOMY AND OF ADRENAL CORTICAL EXTRACT ON DCA-HYPERTENSION IN THE RAT<sup>1</sup>

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IT IS a well documented fact that desoxycorticosterone acetate (DCA) is capable of producing an elevation of the blood pressure in a wide variety of species (1). It seems equally certain that the adrenal cortex normally liberates some product or products identical in action with this synthetic steroid (2). In our investigations concerning the action of this compound in the rat we were able to demonstrate not only its potency in the intact animal (3) but also its immediate effect on the kidney (4).

The present study was undertaken to determine whether the adrenal gland might normally liberate some factor opposed to DCA in its action. This idea was first suggested by the observation in the dog (5) and the impression in man (1) that pressor effects seemed more readily elicited by DCA in the absence of normal functioning adrenal cortical tissue. Soffer (1) has stated that this "would suggest that in addition to the hypertensive factor manufactured by the adrenal cortex an additional blood pressure balancing factor is similarly produced," yet little direct investigation of this problem has been undertaken. The earlier work referred to above does not solve the problem for it was only indirectly concerned with the question. Thus, Swingle *et al.* (5) noted that the degree of pressure rise following DCA administration was greater in adrenalectomized than in intact dogs. Since the adrenalectomy was performed some time prior to the DCA administration, however, the blood pressure base line in these animals was lower to start with, although there was no significant difference in the final pressures obtained. A similar criticism applies to the observations in Addisonian patients in whom the duration of the disease represents an uncontrolled variable. The only suitably controlled report seems to be that of Green (6) who was able to demonstrate a minimally increased sensitivity to DCA in adrenalectomized rats only if they were given small doses of the steroid.

Along similar lines, Pines *et al.* (7) have considered the possibility of depressing adrenal cortical function as a possible means of decreasing the blood pressure of hypertensive patients. Continued administration of adrenal cortical extract for one month decreased the 'resting' blood pressure in 3 of 4 hypertensive subjects. Allowing the possibility of a non-specific effect, these authors felt that the result they obtained might represent either "suppression of adrenal cortical function or counteraction of a desoxycorticosterone-like pressor hormone."

Received for publication January 24, 1949.

<sup>1</sup> Aided by a grant from the Dazian Foundation for Medical Research.

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From the above, it seemed of obvious importance to determine whether the adrenalectomized animal was, in fact, more sensitive to DCA before accepting the thesis for an adrenal cortical anti-pressor secretion. Similarly, it appeared of importance to establish by administering cortical extract to animals with DCA hypertension whether the effects observed by Pines *et al.* (7) could have been due to depressor material in the extract.

#### EXPERIMENTAL

Three separate experiments were performed to determine the relative sensitivity of the adrenalectomized rat to DCA administered in pellet form and a 4th to determine the effect of cortical extract administered to DCA-treated animals.

*Experiment 1.* Thirty-three male albino rats of the Sherman strain were maintained for a period of 44 days. *Group 1*, consisting of 8 animals, served as untreated controls. *Group 2*, consisting of 14 animals, received a subcutaneous implant of DCA (one third of a 75-mg. Schering Cortate pellet) on the 1st, 16th and 33rd days

TABLE I

TREATMENT	CONTROL	DCA-SALINE	ADRENALECTOMY + DCA-SALINE
Initial wt., gm.	130	124	125
Final wt., gm.	170	177	195
Blood pressure, mm. Hg 4th week	96 $\pm 14$	107 $\pm 14$	119 $\pm 16$ p <0.05
7th week	105 $\pm 9$	122 $\pm 19$	125 $\pm 14$
Heart wt., mg/100 cm. <sup>2</sup>	150	171	200
Kidney wt., mg/100 cm. <sup>2</sup>	358 $\pm 13$	476 $\pm 13$	501 $\pm 9$
			$\pm 23$

of the experiment. The 11 animals of *group 3* were adrenalectomized on the first day of the experiment and were subsequently treated in the same way as those of *group 2*. Saline (1%) was substituted for the drinking water of both DCA-treated groups. Blood pressure was determined by the method of Byrom and Wilson (8) using ether as anesthetic, on the 25th, 29th and 31st days and again on the 44th day of the experiment. At the conclusion, hearts and kidneys were weighed after 24 hours' fixation. Table I presents the findings. Since the three separate blood pressure determinations in the 4th week yielded the same result they are aggregated as a single datum in the table.

As has previously been reported (9) rats of the Sherman strain are rather resistant to the pressor effects of DCA. Nonetheless, it is quite apparent that in the earlier period (4th week) the adrenalectomized animals are slightly more sensitive to the pressor effect of DCA than are the non-adrenalectomized, in this aggregate datum. This difference disappears in the 7th week. At the end of the experiment, heart and kidney weight was increased in both DCA-saline treated groups, the increase being greater in the adrenalectomized animals.

*Experiment 2.* In order to obviate the possibility that the substitution of saline for drinking water in the previous experiment had been an additional complication, a second experiment was undertaken in which DCA alone was administered to adrenalectomized and intact animals. Three groups of 8 male albino rats of an inbred Wistar strain were maintained for 26 days. *Group 1* served as untreated controls, *group 2* received DCA implants (one third of a 75-mg. pellet) on the 1st, 5th, 10th, 15th and 20th days and *group 3* received the same treatment as *group 2* but these animals were bilaterally adrenalectomized on the first day of the experiment. Blood pressure was determined at weekly intervals by the method already described. At the conclusion of the experiment the animals were killed and hearts and kidneys weighed after 24 hours' fixation. The data are presented in table 2.

TABLE 2

TREATMENT	CONTROL	DCA	ADRENALECTOMY + DCA
Initial wt., gm.	70	94	98
Final wt., gm.	158	180	185
Blood pressure, mm. Hg			
7 days	95 ±11	125 ±16	103 ±10
13 days	101 ±6	124 ±13	122 ±12
19 days	96 ±9	127 ±20	134 ±21
26 days	89 ±13	117 ±20	134 ±13
Heart wt., mg/100 cm. <sup>2</sup>	181 ±9	215 ±20	235 ±11
Kidney wt., mg/100 cm. <sup>2</sup>	453 ±51	511 ±73	520 ±50

Although there was a suggestion that the adrenalectomized animals were again more susceptible to the pressor effect of DCA in the 4th week, the difference between the two DCA-treated groups is not significant. Kidney and heart weights were increased to the same degree in both groups. It is apparent that when no saline is administered there is no real difference between the sensitivity of intact and adrenalectomized rats to the pressor effects of DCA. It is, of course, possible that if a larger series of animals were tested the suggested difference between groups might actually become significant.

*Experiment 3.* The preceding two experiments taken together had shown that any slight increase in the susceptibility of adrenalectomized animals to the pressor effects of DCA was evident only when saline was substituted for the drinking water and then only in an early phase of the treatment.

It had also been noted that, following treatment with DCA and saline, adrenalectomized animals showed a greater increase in kidney weight. A third experiment was now undertaken in Wistar animals to study this renal effect and to confirm the

previous findings with regard to blood pressure. Treatment was carried out for 38 days in three groups of 10 animals. *Group 1* consisted of intact controls, *group 2* of DCA-treated intact animals and *group 3* of DCA-treated adrenalectomized rats. One per cent saline was substituted for the drinking water of the DCA-treated animals and a record was kept of the water intake of the three experimental groups. DCA pellets in this experiment were implanted on the 1st, 14th and 28th days.

TABLE 3

TREATMENT	CONTROL	DCA-SALINE	ADRENALECYTOMY + DCA-SALINE
Initial wt., gm.	61	72	64
Final wt., gm.	204	178	174
Blood pressure, mm. Hg	100	157	126
35 days	±12	±33	±18
37 days	102	149	130
Heart wt., mg/100cm. <sup>2</sup>	±27	±35	±32
Kidney wt., mg/100cm. <sup>2</sup>	492	648	710
C <sub>IN</sub> cc/min.	0.38	0.37	0.38
C <sub>PAH</sub> cc/min.	3.04	3.30	3.12
Tm <sub>PAH</sub> mgm/min.	0.137	0.170	0.154
C <sub>IN</sub> cc/min.	0.81	0.59	0.55
C <sub>PAH</sub> cc/min.	6.45	5.37	4.10
Tm <sub>PAH</sub> mg/min.	0.315	0.275	0.223
FF as %	13.04	11.1	13.03
C <sub>PAH</sub> /Tm <sub>PAH</sub>	±3.2	±2.0	±3.9
Water intake cc/day/100gm.	21.2	20.1	20.0
1st week	28	39	35.5
2nd week	24	34	39
3rd week	23	36	35
4th week	19	35	30.5
5th week	17	41	28.5

Renal function was determined at the conclusion of the experiment according to methods previously described (10). Following this procedure, the animals were killed and the kidneys and hearts weighed after 24 hours' fixation. Table 3 presents the data.

It is again apparent that adrenalectomized animals are not more susceptible to the pressor effects of DCA; indeed, blood pressure was significantly lower than in the intact DCA-treated group. Although renal function remained normal in both DCA-treated groups, renal hypertrophy did occur, so that renal function per gram of

kidney tissue was considerably reduced. Renal hypertrophy was greater in the adrenalectomized than in the intact animals, despite the fact that in the latter part of the experiment their saline intake declined considerably.

*Experiment 4.* Thirty-six male albino rats of an inbred Wistar strain were divided into three equal groups and maintained for 28 days. *Group 1* served as untreated control while *group 2* received DCA by the subcutaneous implantation of a pellet (one third of a 75-mg. pellet) on the 1st, 6th, 11th, 16th and 21st days of the experiment. The animals of *group 3* received DCA in the same manner as those of *group 2* but for the duration of the experiment were given daily injections of cortical

TABLE 4

TREATMENT	CONTROL	DCA	LIPO-CORTICAL EXTRACT + DCA
Initial wt., gm.	52	49	53
Final wt., gm.	175	153	159
Blood pressure, mm. Hg			
14 days	104 ±12	117 ±10	124 ±12
23 days	101 ±14	122 ±19	128 ±13
27 days	96 ±17	133 ±14	127 ±14
Heart wt., mg/100cm <sup>2</sup>	180 ±19	23 ±14	205 ±17
Kidney wt., mg/100cm <sup>2</sup>	459 ±65	534 ±25	535 ±45
C <sub>IN</sub> cc/100cm <sup>2</sup> /min.	0.32 ±0.06	0.28 ±0.05	0.31 ±0.07
C <sub>PAH</sub> cc/100cm <sup>2</sup> /min.	2.82 ±0.36	2.91 ±0.26	2.83 ±0.40
Tm <sub>PAH</sub> mgm/100cm <sup>2</sup> /min.	0.173 ±0.022	0.174 ±0.024	0.161 ±0.017
FF as %	11.4 ±1.9	9.54 ±1.9	11.0 ±1.8
C <sub>PAH</sub> /Tm <sub>PAH</sub>	16.3 ±0.5	16.7 ±1.0	17.6 ±2.1

extract. For this purpose,  $\frac{1}{16}$  cc. of Upjohn Lipo-Cortical extract was injected subcutaneously once daily for 5 days each week. Each cc. of this extract contains 40 rat units by biological standardization, equivalent to 2 mg. of 11 dehydro 17 hydroxycorticosterone (Kendall's compound E). The choice of a daily administration of  $\frac{1}{16}$  cc. (4 RU or 0.2 mg. Kendall's E) was determined by the amount of material available for this work. At the end of the third week, when it became apparent that the extract had not modified the hypertensive effect of the DCA, the dose of extract was doubled for the 4th and last week of the experiment.

Blood pressure was determined on the 14th, 23rd and 27th days of the experiment. On the 28th day renal function was estimated. Table 4 presents the pertinent data.

Aside from confirming findings which have previously been reported, such as the effectiveness of DCA in producing hypertension even without any intensifying measures, and the absence of renal functional change during the earlier stages (4 weeks) of such treatment, this experiment is distinguished by its complete negativity. The lipocortical extract, in the amounts given, in no way modified either the blood pressure or renal functional status of animals treated with DCA for 4 weeks. These observations are confirmed by both kidney and heart weight which are reasonably sensitive indicators of interference with renal function on the one hand or elevation of blood pressure on the other. The experiment again demonstrates that the maintenance of normal renal function in DCA-treated animals requires an increase in kidney mass.

#### DISCUSSION

*Adrenalectomy.* These experiments have demonstrated the difficulty of establishing the relative sensitivity of adrenalectomized animals to the pressor effect of DCA. A slightly increased susceptibility was observed in the early phase of treatment but this appeared as a significant finding only when saline was administered with the DCA. No increase in sensitivity was elicited in the later phases of treatment (6th and 7th weeks) despite the administration of saline as drinking water. A greater degree of renal hypertrophy was observed, however, in adrenalectomized animals given DCA with saline for this longer period.

These findings are in agreement with those of Green (6) who was able to show only a slight increase in the blood pressure response of the adrenalectomized rat given DCA with saline, but not with the indirect observation in the dog (5) and man (1). It seems reasonable to suggest that the minimal early sensitivity observed only when salt is given with the DCA hardly indicates a specific anti-pressor activity for the adrenal cortex. Rather it would seem that the effects observed can more readily be ascribed to the general metabolic disturbance following adrenalectomy.

While these findings argue against the liberation of specific depressor substances from the adrenal cortex they are in no way conclusive. Exogenously administered DCA might easily suppress such cortical depressor functions in the intact animal to begin with, so that by the criteria used here no difference could be expected between intact and adrenalectomized animals. Similarly, a homeostatic mechanism satisfactory for normal needs might be overshadowed, if not actually depressed, by the quantities of pressor material here added to the organism as exogenous DCA. Nonetheless, the experiments do indicate that if materials capable of counteracting DCA do exist in the organism they must be of a more subtle nature than has previously been indicated in the literature.

*Cortical extract.* Under these circumstances, it is not surprising that the gross procedure of injecting adrenal cortical extract to DCA-treated animals was entirely without effect. If materials capable of counteracting the DCA hypertensive effect are present in such an extract, their subtle presence is masked, not only by those corticoids with no effect on the blood pressure, but by the liberal content in such extracts of substances with DCA-like action. It seems apparent, then, that the

depressor action observed by Pines *et al.* (7) in hypertensive patients could hardly have been due to a direct action of the material itself. This conclusion would underline the alternate explanation of these authors that the extract suppressed adrenal function.

#### SUMMARY

Experiments in intact and adrenalectomized rats revealed only a minimal increase in the sensitivity of adrenalectomized animals to the pressor effect of DCA. This was observed as a significant finding only when sodium chloride was added to the drinking water, and only as a temporary phase during the course of treatment. A greater degree of renal hypertrophy occurred following such treatment in the adrenalectomized rat, but again only in the presence of added saline. These results do not support the concept of a specific antipressor activity in the normal adrenal gland, but rather reflect the precarious water balance of the adrenalectomized animal. The administration of adrenal cortical extract to DCA-treated animals did not modify the effects of this latter substance, indicating the absence of any grossly detectable depressor substance in the extract used.

The authors wish to thank Dr. W. Stoner and Mr. W. E. Fielding of the Schering Corporation for the Cortate pellets and Dr. H. F. Hailman of the Upjohn Co., for the Lipo-Adrenal Cortex used in this work. Sodium para-aminohippurate was supplied through the courtesy of Dr. W. Boger, Sharp and Dohme, Inc.

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# SPREAD OF EXCITATION IN THE DOG HEART<sup>1</sup>

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**S**EVERAL methods have been employed in the past to determine the time of arrival of the excitation wave at various points on the surface of the dog heart (1-4). There is general agreement that the earliest point on the ventricular surface to become electronegative is the mid-portion of the anterior right ventricle near the septum. There is further agreement that most of the right ventricular surface becomes depolarized from 8 to 10 milliseconds earlier than the left ventricular surface. Most of the methods employed, however, were carried out on animals with exposed hearts, a technique which introduces at least two complications in the proper interpretation of the results: 1) in the exposed heart normal conducting tissues are removed from the surface of the heart, and 2) most of the experiments were done under the cooling effect of room air. In view of the fact that lead II is used as a standard of reference for the measurement of the time of arrival of the excitation wave at the various points on the heart surface, an alteration in lead II caused by these two factors might introduce errors in the measurements because the earliest deflection in the ventricular complex in lead II may not represent the onset of ventricular excitation. Furthermore, if the surface of the heart is cooled by exposure to room air, conduction from the endocardium to the epicardium may be slowed in the exposed area so that the results thus obtained may not correspond to the true order of excitation in the intact animal. In spite of these objections, much useful information has been afforded by such experimentation. However, the methods do not readily lend themselves for study of the effect of such agents as anoxia and drugs on the spread of excitation in the intact animal.

A method for the study of the spread of excitation in the intact animal became available as a result of studies on the nature of unipolar extremity leads in the dog (5-7). In these studies it was found that from an analysis of the QRS complex in any unipolar lead one could ascertain what parts of the heart were being depolarized from moment to moment; this information was afforded by the discovery that preponderant depolarization in the proximal zone of any unipolar lead resulted in downward movement of the beam, while upward movement was caused by preponderant depolarization in the distal zone. When the excitation involved the intermediate zone of the lead or was of equal degree in both proximal and distal zones, the beam remained at the iso-electric line. Since the distribution of the proximal, intermediate, and distal zones for each of the unipolar extremity leads was also established, localization of the

Received for publication February 23, 1949.

<sup>1</sup> Aided by grants from the Fluid Research Fund, Yale University School of Medicine, and from the U. S. Public Health Service.

<sup>2</sup> Senior Research Fellow, U. S. Public Health Service.

excitation process to various parts of the heart became possible. When analyzing a single unipolar lead, however, the excitation process can be localized only to rather large segments of the heart. When the three unipolar leads, VR, VL, and VF, are recorded simultaneously and an analysis is made of the moment-to-moment beam position in each of the leads, then the excitation process can be localized to much smaller segments of the heart. Figure 1 shows that because of the overlapping zones of the three unipolar extremity leads, the various segments of the heart bear a definite relation to each of the three leads. Depolarization in each of these segments results in a specific combination of beam movements in the three leads. For example,

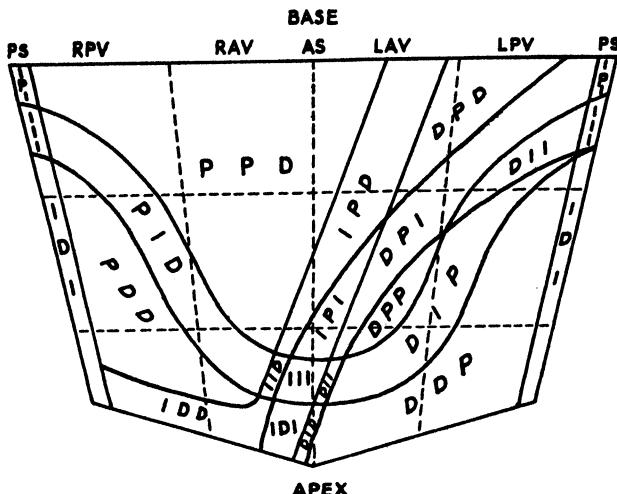


Fig. 1. SCHEMATIC DRAWING OF VENTRICULAR SURFACE OF DOG HEART showing overlapping of the proximal, intermediate and distal zones of leads VR, VL, and VF (composite picture of findings of 40 dogs). PS, posterior septum; RPV, right posterior ventricle; RAV, right anterior ventricle; AS, anterior septum; LAV, left anterior ventricle; LPV, left posterior ventricle; P, proximal zone; I intermediate zone; D, distal zone. The various areas diagrammed are labelled according to their zonal representation in leads VR, VL, and VF. The first of the three letters which label each of the areas represents the zone of lead VR, the second letter the zone of lead VL, and the third letter the zone of lead VF (e.g., area labelled PPD indicates that this portion of the heart lies in the proximal zones of leads VR and VL, but in the distal zone of lead VF).

if the beam position in each of the three leads at a given moment indicates preponderance of depolarization in the proximal zone of lead VR and lead VL (downward movement of the beam in VR and VL) and in the distal zone of lead VF (upward movement of the beam in lead VF) the site of preponderant depolarization is localized to that segment of the heart labelled PPD in figure 1 (the upper anterior right ventricle). If at a given moment in the QRS complex the beam has moved upward in VR and VL (indicating preponderance of depolarization in the distal zones of VR and VL) and at the same time has moved downward in lead VF (indicating preponderance of depolarization in the proximal zone of VF), the side of preponderant depolarization is thus localized to segment DDP in figure 1 (the left apical region). In this manner every combination of beam movements found in the three leads will help to localize

the excitation process to one or another of the various heart segments depicted in figure 1.

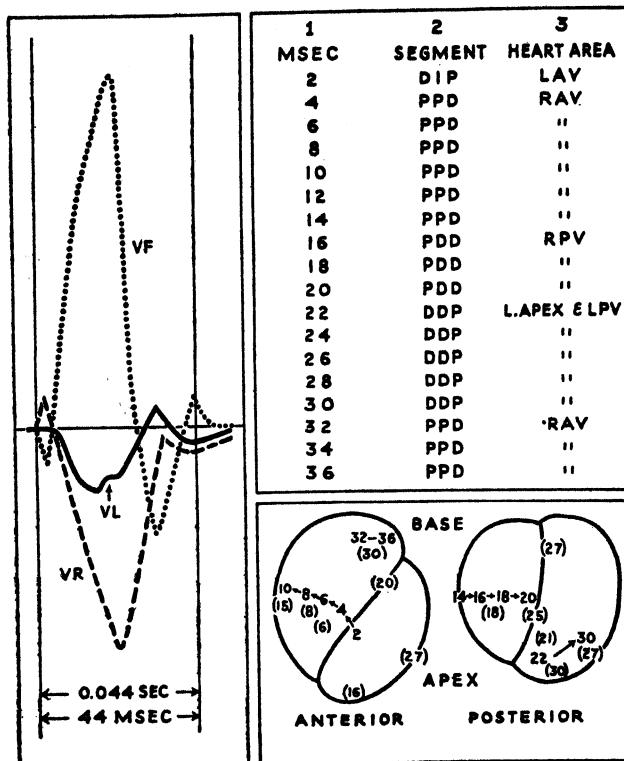


Fig. 2. ANALYTICAL METHOD OF DETERMINING SPREAD OF EXCITATION IN VENTRICLE. VR, VL, and VF are recorded simultaneously and superimposed. The position of the beam in the 3 leads is determined from moment to moment throughout the ventricular complex. MSEC—time in milliseconds during QRS interval; SEGMENT—the three letters P, I, D mean proximal, intermediate, and distal. For each time interval the first of the three letters represents the zone of lead VR in which the excitation is present; the second letter the zone of lead VL, and the third letter the zone of lead VF. Thus as 2 milliseconds segment DIP means that excitation is present in the distal zone of VR, the intermediate zone of VL, and the proximal zone of VF (the lower anterior left ventricle). HEART AREA—area of heart corresponding to segment in column two. LAV, left anterior ventricle; RAV, right anterior ventricle; RPV, right posterior ventricle; LPV, left posterior ventricle. In the lower right-hand corner of the diagram the anterior and posterior surfaces of the dog ventricle are represented. The bold figures are in milliseconds and represent the time of arrival of the excitation process in the various segments of the heart as determined by the analytical method. The figures in parentheses represent the time of arrival of the excitation wave at various points of the surface of the heart in the same animal (with chest closed) as determined by the direct method of Lewis and Rothschild. Note close correspondence between values obtained by the two techniques.

By this type of analysis one can detect the site of preponderant depolarization from moment to moment during ventricular excitation. The sequence of this spread of excitation probably corresponds closely to the actual spread of the excitation wave

through the ventricles for the following reasons: *a*) the initial beam movement certainly can be considered to represent the first site activated, *b*) the speed of conduction through both the right and left bundles is probably the same (since it is known that the fibers in each bundle are of the same size). Therefore preponderance of depolarization in any segment must mean that the excitation wave has reached this area in advance of other areas, for otherwise there would not be enough depolarized fibers to produce the specific movement of the beam, and *c*) as will be seen below, the order of preponderant depolarization corresponds closely to the actual arrival of the excitation process at the surface of these segments.

#### METHOD

The ventricular complexes in the three unipolar extremity leads VR, VL, and VF are recorded simultaneously on a Sanborn Tribeam at rapid speeds (75 mm/sec.) in order to make the measurements technically easier. These complexes are then photographically enlarged and transposed to graph paper. This makes it possible to subdivide the QRS complex into intervals of 4 milliseconds and then to record the position of the beam (as going up or going down) in each of the three leads at successive 4-millisecond intervals. The beam position in each lead is labelled by the letters *P*, *D*, or *I*, depending upon the direction of the beam deflection at each moment. If the beam is being deflected downwards, the letter *P* is used to signify preponderance of depolarization at that moment in the proximal zone of the lead. If the beam is being deflected upwards in a lead, the letter *D* is used to signify preponderance of depolarization in the distal zone of the lead. If the beam remains at the iso-electric line at the onset of ventricular excitation, the letter *I* is used to indicate depolarization in the intermediate zone of the lead. The resulting combination of three letters identifies that segment of the heart which is the site of preponderant depolarization at each successive time interval. The time in milliseconds needed for the excitation wave to involve the various segments of the heart is charted on a diagram of the anterior and posterior surface of the heart (fig. 2).

#### RESULTS

When this technique was applied to the electrocardiographic complexes obtained in a series of 30 dogs it was found that the usual order of segment depolarization was as follows:

TIME IN MSEC. (AFTER ONSET VENTRICULAR EXCITATION)	SEGMENT (IN FIG. 1)	HEART AREA
3	DIP, IPD, or PPD	anterior septum or anterior right ventricle near septum
6-12	PPD	upper anterior right ventricle
15	PDD	posterior right ventricle
18-27	DDP	left apex and posterior left ventricle
30-33	PPD	upper anterior right ventricle (pulmonary conus)

It was observed that beyond 33 to 35 milliseconds occasionally the beam remains above the iso-electric line in all three unipolar extremity leads. Since no segment of the heart has been found to lie in the distal zone of all the leads, localization of the excitation process at such points in the QRS complex is not possible. During this period there may be repolarization of some parts of the heart while depolarization is not yet complete in other parts, resulting in potentials of mixed origin so that the particular beam position can no longer be used in localizing the segment being de-

polarized. It is also possible that the potentials of depolarization at this time at each electrode, though present, are of such low magnitude that they approximate the potential of the central terminal so that the resulting beam positions might be due to the potential variations of the central terminal rather than to the variations of the exploring electrode.

The first region depolarized usually is the mid-anterior septum or the anterior right ventricle. This is followed in turn by the posterior right ventricle, and then by the left apex and posterior left ventricle. In many hearts terminal excitation again involves segment PPD (fig. 2). Since most of this segment was depolarized early, the terminal excitation probably involves the pulmonary conus which is part of the segment.

In order to test the validity of this analytical technique of determining the spread of excitation in the intact animal, the time of arrival of the excitation wave at various points on the heart surface was then determined in four of the same animals by the direct method of Lewis and Rothschild (1). The results obtained by this method were compared with the results obtained on the same animals before their chests were opened by the analytical method described above. In utilizing the method of Lewis and Rochschild, tiny pin electrodes were inserted through the pericardium at various points in such a manner that the electrode was in contact with the epicardium without injuring it. The indifferent electrode consisted of a central terminal electrode constructed as described previously (5). The lungs were restored to their normal position, the chest closed tightly, and the animal allowed to breathe on his own. During the exposure of the heart, a lamp was allowed to shine over the operative field to prevent undue cooling of the anterior heart surface, and records were taken only after a period of an hour or so had elapsed after closure of the chest. It had been found in previous experiments (8) dealing with heart temperature that the normal temperature of the anterior surface of the heart is not restored until at least one hour after closure of the chest wall. These precautions were taken to minimize any possible effects of lowered heart temperature upon the spread of conduction and depolarization (9). Comparison of the results obtained by the two techniques on the same animal reveal close correlation (fig. 2).

#### DISCUSSION

The spread of excitation in the dog heart as determined by the analytical method described above is found to correspond fairly closely with the spread of excitation as determined by Lewis and Rothschild and others. It can be seen that excitation first involves the anterior septal region and extends rapidly to depolarize the anterior and posterior right ventricle before most of the left apex and posterior left ventricle become depolarized. It should be noted that points on the surface of the left lateral ventricle receive the excitation wave later than do corresponding points on the right lateral ventricle. If one assumes that the subjacent endocardial points in the right and left ventricles are probably at the same distance from the A-V node, and that the speed of conduction along both bundles is the same—both reasonable assumptions—then the later arrival of the excitation wave at the surface of the left ventricle could be attributed to the greater thickness of the left ventricle. Lewis and Rothschild considered that by their technique they were measuring the time of arrival of the

excitation wave at the various points on the surface of the ventricles and that the later arrival at the surface of the left ventricle was indeed due to the greater thickness of this ventricle (1). Using the analytical method, the time of arrival of the excitation wave at various segments of the heart, rather than at specific surface points, has been determined. Nevertheless, the results of the two methods yield comparable values, suggesting that what is being measured by the analytical method is also depolarization at the epicardial surface of the various segments. If this be so, one can conclude that surface depolarization dominates the formation of the electrocardiogram in leads recorded from the surface of the body, a view already expressed by several authors (10, 11).

The ventricular complexes in the three unipolar extremity leads of most dogs tend to resemble each other very closely. However, there are occasionally animals that exhibit somewhat different complexes. Since these changes in the form of the ventricular complexes can be due to slight alterations in the position of the heart in these animals as compared to the average animal, one is not justified in concluding that the difference in the complexes indicates a different sequence of excitation, although this possibility cannot be excluded. These variations in the complexes of different animals do not, however, invalidate the use of the method in studying the effect of various agents upon the spread of excitation in the intact animal since each animal serves as his own control.

#### SUMMARY

The spread of excitation in the dog ventricle has been determined in the intact animal by an analysis of the ventricular complexes simultaneously recorded in the unipolar extremity leads VR, VL, and VF. The method depends upon the fact that depolarization in different parts of the heart produces specific combinations of simultaneous beam movement in the three unipolar extremity leads. An analysis of the beam positions from moment to moment in the QRS complex in each lead permits the recognition of the time when depolarization occurs in the various segments of the ventricles, and, therefore, the time of arrival of the excitation wave at the various segments. The results obtained by this method correspond closely to those obtained in the same animals when the direct method of Lewis and Rothschild is employed.

The order of excitation is usually as follows: mid-anterior septum, anterior right ventricle, posterior right ventricle, left apex and posterior left ventricle, and pulmonary conus.

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# EXTRACELLULAR WATER CONTENT OF THE HEART IN DOGS SUBJECTED TO HEMORRHAGIC SHOCK MEASURED WITH THE RADIOACTIVE ISOTOPE OF SODIUM<sup>1,2</sup>

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**W**IGGERS suspected the existence of a cardiac factor in the irreversible shock produced by his technique of hemorrhagic hypotension because he observed a progressive fall in cardiac output at a time when venous return and arterial pressure remained adequate (1).

Altered capillary permeability is a prominent feature of myocardial injury in a number of circumstances. The human heart is edematous in failure (2). Hearts which have failed in isolated heart preparations also exhibit edema (3). Regions of the ventricle made temporarily ischemic by experimental occlusion of a coronary artery have been found to contain an excess of extracellular water after as short a period of occlusion as five minutes (4). Asphyxia and anoxia produce profound alterations in the myocardial capillaries (5, 6). It therefore seemed possible that myocardial capillary injury might occur during the severe hypotension to which dogs are subjected in the Wiggers procedure. If this were the case, the myocardium might become edematous and the edema itself cause progressive failure of the heart for purely physical reasons.

The technique introduced by Manery and Bale (7) for measurement of the so-called 'sodium space' makes investigation of the volume of the extracellular fluid relatively simple. We therefore applied this technique to determine whether myocardial edema does develop in dogs subjected to the Wiggers procedure.

## METHODS

The dogs used in this study were unselected mongrels and were for the most part well nourished and well hydrated. Anesthesia was obtained by using 125 to 150 milligrams of sodium barbital and 3 milligrams of morphine sulfate per kilogram intravenously. No experiment was begun less than one hour following the injection, and fairly uniform anesthesia was obtained. Heparin was used as an anticoagulant, and we were unable to see that it was a significant factor in the production of any pathological change.

In the production of 'hemorrhagic shock' in dogs, we successfully reproduced the procedure described by Wiggers *et al.* (8). Fifteen dogs were used to develop facil-

Received for publication February 21, 1949.

<sup>1</sup> This work was supported in part by research grants from the Division of Research Grants and Fellowships of the National Institute of Health, U. S. Public Health Service, The Eli Lilly Company and Ciba Pharmaceutical Products, Incorporated.

<sup>2</sup> Published with permission of the Chief Medical Director, Department of Medicine and Surgery, Veterans Administration, who assumes no responsibility for the opinions expressed or conclusions drawn by the author.

ity with this technique (*Series A*). A similar series of 9 dogs (*Series B*) received radioactive sodium. The isotope was supplied as sodium carbonate. Prior to administration it was converted to isotonic sodium chloride and the *pH* adjusted to 7.4 by the addition of hydrochloric acid. After this the solution was carefully administered intravenously. At least one hour was allowed for the isotope to equilibrate in the sodium space.

When the animal died or was killed, whole blood was drawn under mineral oil, and three samples of ventricular muscle were excised from the heart, which was often still beating. All possible blood was blotted from the sections, using gauze, and the chordae tendineae, fat and valves were removed from the tissue. One portion of the tissue was dried to constant weight in order to determine the 'total water' of the myocardium.

To calculate the extracellular and intracellular water according to the method of Manery and Bale (7), it was necessary to determine the relative concentration of radioactive sodium in the heart muscle and in the serum.

Samples of heart muscle were prepared for counting by two different methods. The first procedure involved extraction of the radioactive sodium. A sample of heart muscle was weighed, water added to bring the total weight to 200 grams, and the mixture macerated in a Waring Blender for 20 minutes. The resulting mixture was centrifuged at 3500 rpm for 30 minutes. One milliliter samples of the supernatent fluid were placed in cups and dried slowly for 45 minutes in an oven. Then their activities were determined on a Geiger-Mueller Counter.

In the second procedure, duplicate samples of heart muscle were prepared for counting by dry ashing the tissue overnight in a muffle furnace at 500 to 600° C. The activity of the ash was then determined. Satisfactory checks were obtained on the activity of duplicate ashed samples. The activity of the ashed heart muscle was in all cases slightly higher (3-10%) than the activity of water-extracted heart muscle. Dry ashing was then adopted as the method of choice because of its efficiency and convenience. As a further check on 'background counts' samples of heart muscle were also taken from normal untreated dogs, but no natural radioactivity could be detected in these samples.

The whole blood obtained was centrifuged, and 1 ml. samples of serum placed in cups, dried and counted. This procedure gave uniform samples for counting, and the activity of duplicate samples checked closely.

Each sample container was counted several times and these counts were averaged as the count of the sample. This was compared with a duplicate sample treated in the same manner. The standard deviation of the difference between these two estimates was determined and corrected for sample size. In the case of the heart muscle extracts, in 95 per cent of the cases the difference between duplicates was less than 11 counts per minute and the error of the mean of two such duplicates was 8 counts per minute. Over the range of activities observed in these extracts, this represents an error of about 8 per cent. In the case of the ashed heart samples the corresponding figure was about 5 per cent and for serum samples it was even less.

The extracellular water of the ventricular myocardium was calculated according to the method of Manery and Bale (7), but no correction was applied for the Gibbs-

Donnan equilibrium or for the volume occupied by the protein of the serum. We felt that no such correction was justified because the effects tended to cancel each other in the case of sodium. Furthermore, Kaltreider and Meneely (9) were unable to find systematic differences between the activity of serum and the activity of transudates. We have followed the symbols used by Manery and Bale (7);  $(H_2O)_E$ , designates extracellular water;  $(H_2O)_I$ , intracellular water, and  $(H_2O_T)$ , total water. All these values were expressed as percentage of the wet weight of the ventricular muscle.

It was further necessary to know the activity of the erythrocytes, since 107 milliequivalents per liter of sodium are found normally in such cells in the dog. It was possible to compute the activity of the red cells by determining packed cell volume, the activity of whole blood, and the activity of serum. This was done in a series of 6 control and 7 shock dogs.

Eleven dogs (*Series C*) were used as controls. They were anesthetized in the same manner as the shock dogs, were given radioactive sodium intravenously and in an average of 1 hour and 5 minutes were bled from an artery, the beating heart exercised and samples of serum and heart muscle treated as under *Series B*.

Six dogs (*Series D*) served as negative controls and were given large amounts of saline intravenously. The animals were anesthetized and given radioactive sodium intravenously. From a standard infusion set at a height of about 3 feet above the level of the auricle normal saline solution was permitted to flow as fast as the apparatus would permit until the animals began to show some ill effects, such as abdominal distension, cyanosis, cardiac irregularity, or dyspnea. The infusion was then halted for intervals ranging from 30 minutes to an hour and then continued in the same manner until the animal's death.

Blood and myocardial samples were obtained and treated as were similar samples under *Series B*. Complete routine autopsies were performed on all dogs. The erythrocytes contributed from 11 to 45 per cent of the activity of whole blood. The activity contributed by the red cells was directly related to the packed cell volume. In all cases there was hemoconcentration in shock dogs, and therefore the activity of whole blood as compared with that of serum was relatively higher in shock dogs than in the control animals. Actual determinations of hemoglobin residual in ventricular muscle samples were made in 6 dogs. These data showed that residual blood in such samples contributed so little activity to the sample that it was less than the error involved in the counting. Therefore, we felt justified in basing the calculation of  $(H_2O)_E$  and  $(H_2O)_I$  on the activity of serum rather than that of whole blood.

#### RESULTS

The distribution of water in the myocardium in *Series B*, *C*, and *D* is presented in table 1. It is at once evident that there is no increase of myocardial extracellular water in dogs subjected to the Wiggers procedure (8) of hemorrhagic hypotension to induce irreversible shock. The mean extracellular water content of  $24.7 \pm 4.4$  per cent of wet weight of tissue in these shock dogs is not different from the mean of  $23.5 \pm 4.5$  per cent present in controls. The control value is very similar to the data of Manery and Bale (7). The method is adequate for detection of edema as is shown

by the negative control dogs where the mean extracellular water content in the myocardium was  $33.5 \pm 3.6$  per cent. This is significantly different from the positive control series.

#### DISCUSSION

The concept of developing cardiac edema as an explanation of the deterioration of the heart in irreversible shock was an attractive one. In a preliminary presentation (10) we actually suggested this possibility. Our hypothesis was certainly not borne out by the experiments reported above. It is evident that a subtler cause for myocardial deterioration in irreversible shock must be sought.

TABLE I

Series B DOGS SUBJECTED TO WIGGERS' PROCEDURE			Series C POSITIVE CONTROLS (NO HYPOTENSION)			Series D NEGATIVE CONTROLS (SALINE INFUSION)					
No.	(H <sub>2</sub> O) <sub>E</sub>	(H <sub>2</sub> O) <sub>I</sub>	(H <sub>2</sub> O) <sub>T</sub>	No.	(H <sub>2</sub> O) <sub>E</sub>	(H <sub>2</sub> O) <sub>I</sub>	(H <sub>2</sub> O) <sub>T</sub>	No.	(H <sub>2</sub> O) <sub>E</sub>	(H <sub>2</sub> O) <sub>I</sub>	(H <sub>2</sub> O) <sub>T</sub>
1	22.8	57.8	80.6	1	28.1	49.4	77.6	1	31.6	47.7	79.3
2	31.8	46.7	78.5	2	27.3	49.2	76.5	2	33.1	48.2	81.3
3	32.1	46.9	79.0	3	17.3	64.7	82.0	3	40.1	39.1	79.2
4	26.2	49.2	75.4	4	29.4	49.8	79.2	4	34.9	52.4	87.3
5	20.7	54.3	75.0	5	24.8	57.7	82.5	5	30.4	51.3	81.7
6	23.6	49.9	73.5	6	16.5	55.3	71.8	6	31.0	55.8	86.8
7	21.7	56.9	78.6	7	27.5	47.0	74.5				
8	21.4	55.4	76.8	8	25.8	47.6	73.9				
9	22.2	56.7	78.9	9	19.3	55.9	75.1				
				10	23.4	54.6	78.0				
				II	24.2	55.9	80.1				
	24.7	52.6	77.4		23.5	53.8	77.3		33.5	49.1	82.5
	$\pm 4.4$	$\pm 5.5$	$\pm 4.0$		$\pm 4.5$	$\pm 5.5$	$\pm 3.6$		$\pm 3.6$	$\pm 5.6$	$\pm 5.4$

*Series B* dogs were subjected to hemorrhagic hypotension according to Wiggers' (8) method. *Series C* dogs were treated in a manner identical with *Series B* dogs except that they were not subjected to hypotension. *Series D* dogs were given massive saline infusions to induce edema.

(H<sub>2</sub>O)<sub>E</sub> is extracellular water, (H<sub>2</sub>O)<sub>I</sub> is intracellular water, (H<sub>2</sub>O)<sub>T</sub> is total tissue water: all expressed as % wet wt. of ventricular muscle.

#### SUMMARY

The method of Manery and Bale (7) was employed to measure the extracellular water of the myocardium in 3 groups of dogs treated in different ways: The first group was subjected to severe hemorrhagic hypotension to produce irreversible shock by the technique of Wiggers (8). The second group represented positive controls in that they were treated in the same manner as the shock dogs but were not bled. The third group, which served as negative controls, were given large and rapidly administered intravenous infusions of saline.

The positive control dogs showed an extracellular water content in the myocardium of 23.5 per cent of the wet weight of tissue. This is in quite close accord with the data of Manery and Bale (7). The negative control dogs developed an edema of myocar-

dium obvious on gross and microscopic examination. The extracellular water in these hearts was 33.5 per cent of the wet weight of the tissue. This is significantly and convincingly different from the positive controls. Thus the method is adequate to detect edema when it is present.

The extracellular water of the myocardium of the dogs subjected to Wiggers' (8) hemorrhagic hypotension procedure was 24.7 per cent of the wet weight of tissue. There is, therefore, no edema of the heart in dogs subjected to this form of irreversible shock.

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# VASODEPRESSOR RESPONSES TO MORPHINE FOLLOWING HEMORRHAGIC HYPOTENSION<sup>1,2</sup>

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MORPHINE sulfate is used extensively in cases of post-traumatic and hemorrhagic hypotension to relieve the pain and emotional tension which many patients exhibit. Considerable differences have been encountered in the ability of these patients to tolerate the drug. In many instances, no untoward reactions are discernible; in others the administration of morphine leads to an exacerbation of the symptoms of shock. There would therefore appear to be significant variation in the response to morphine depending on the precise state of shock at the time the morphine is given.

In experimental studies the usual doses of morphine (1-5 mg.) have been found to have no significant hypotensive action in the normal dog (1). When morphine is administered prior to hemorrhage in normal dogs and in dogs during early or impending shock following acute massive hemorrhage, there are no immediate vasodepressor responses demonstrable (2, 3). Systematic investigation has not been made concerning the effects of morphine during the progressive development of a state of shock which becomes increasingly refractory to blood replacement. The present report deals with the vascular responses to morphine when the agent is given during the appearance of the so-called 'irreversible state' of shock in dogs subjected to graded hemorrhage.

Recent studies based on direct observations of the omental capillary bed (4) and bioassay of blood samples (5, 6) have indicated that the syndrome following prolonged hemorrhagic hypotension is divided temporally into three phases: 1) a hyperreactive period, with greatly heightened peripheral vasmotor reactivity, the presence of blood-borne vasoexcitor materials (VEM) and marked vasoconstriction of larger blood vessels; 2) a transitional period, in which the augmented vascular reactivity becomes less pronounced and the blood contains both vasoexcitor and vasodepressor materials (VDM); and 3) a hyporeactive period, characterized by diminished or absent vasmotor activity, vasodilatation, and the predominance of vasodepressor substances in the blood.

After restoring normotensive levels by transfusion during the hyporeactive stage,

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Received for publication January 25, 1949.

<sup>1</sup> This work was carried out in part under the direction of Dr. Robert Chambers while the authors were at New York University in the Laboratory of Cellular Physiology.

<sup>2</sup> The work described in this paper was done under a contract recommended by the Committee on Medical Research between the Office of Scientific Research and Development, New York University and Cornell University Medical College. It was also aided by grants from the Josiah Macy, Jr. Foundation and the Eli Lilly Company.

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the muscular vessels become only partially narrowed. The blood flow progressively improves but remains sluggish on the venous side of the circulation. Arteriolar and precapillary vasomotion does not reappear, however, and the epinephrine reactivity of contractile vessels increases but seldom returns to normal levels. Approximately 90 per cent of these animals succumb within 3 to 10 hours after completion of the transfusion, with a gradual fall in blood pressures and progressive return of peripheral vascular dysfunction. The influence of morphine and certain other agents were determined with specific reference to each of these categories.

Other investigators have injected morphine prior to bleeding (2), or at the end of one to three hours of moderate hypotension (3). In the latter instances, peripheral vasomotor reactivity was presumably still in the hyperreactive period described above, or in the transitional phase. It has been our experience that during this period the animal is still capable of handling vasodepressor substances, in contrast to their inability to do so during the subsequent vasodepressor stage of the syndrome (6). Moreover, morphine administration during the period subsequent to transfusion closely duplicates the situation in clinical shock when this agent is frequently given post-operatively to patients who have been in shock and transfused.

For this reason, most of the experiments reported here deal with the vascular effects of morphine injected intravenously after the prolonged hypotensive period has been terminated by whole blood transfusion. Post-transfusion blood pressures at the time morphine was administered were essentially similar to those of the control unbled animals. This procedure permitted a comparison of the effects of morphine on animals in the control and in the experimental group with comparable blood pressure ranges.

#### METHODS

Hemorrhagic shock was induced in dogs in which the omentum was exteriorized for microscopic study. One group of dogs were anesthetized with morphine sulfate, 2 mg. intravenously or 12 mg. subcutaneously per kg. of body weight. Such doses produce a euphoric state in the dog which does not actually represent a state of true anesthesia. These animals were compared to a control group receiving no general anesthesia. In both groups of animals local procaine anesthesia was employed for cannulating the femoral artery and for exteriorizing the omentum. Also included in the present report are several animals anesthetized with cyclopropane and subjected to hemorrhagic shock. In most instances the omentum was exteriorized and kept both warm and moist by a continuous irrigation with Ringer-gelatin maintained at body temperature. This technique has been described in detail in a previous publication (4). Mean blood pressure was recorded continuously by a mercury manometer from the femoral artery. Morphine was administered intravenously (2-6 mg/kg.) during the shock syndrome. On a second group of hemorrhaged animals, morphine was given intravenously following the restoration of normal blood pressure ranges by blood transfusion at the onset of the hyporeactive state.

#### RESULTS

##### *Unhemorrhaged Animals*

*Local anesthesia.* Fifteen animals (8-15 kg. in weight) were prepared by using a 1.0 per cent procaine solution to produce abdominal field block and the omentum exteriorized for microscopic study. No other medication was given. Blood flow was found to be rapid throughout the arteriolar and venular systems. Spontaneous vasomotion of the terminal arterioles and precapillary sphincters was active and was predominantly in the 'closed' or constricted phase. Capillary blood flow was inter-

mittent and asynchronous. The threshold response of the terminal smooth muscle components of the bed to topically applied epinephrine occurred with concentrations of one part in five to ten million. The arterial blood pressure averages 128 mm. Hg  $\pm$  10 mm.

*Morphine anesthesia.* Morphine sulfate, 2.0 mg/kg., was slowly injected intravenously (over a 15-20-second period) into 14 dogs. An equal number were given 12 mg. morphine/kg. subcutaneously. The omentum was then exposed following local infiltration with procaine and examined under the microscope.

There were no pronounced differences in the peripheral circulation of the omentum of these animals as compared to that of the controls (table 1). Spontaneous vaso-motor activity was in general as frequent and of a duration similar to that in the unmorphinized dogs. Arteriolar tone and peripheral blood flow was comparable in both groups. The epinephrine reactivity of the terminal arterioles and precapillaries in animals receiving morphine was slightly below those of the control dogs (table 1).

TABLE I. OMENTAL CIRCULATION IN CONTROL DOGS BEFORE BLEEDING

ANESTHESIA	NO. OF DOGS	B.P. MM. HG	BLOOD FLOW	VASOMOTION	TONE OF VESSELS	EPINEPHRINE REACTIVITY <sup>2</sup>
None <sup>1</sup>	15	128	Intermittent, rapid	Prominent	Arterioles and ven- ules narrow	1:8,000,000
Morphine sulfate (2-12 mg/kg.)	28	120	Intermittent, rapid	Slightly exag- gerated	Arterioles narrow; venules slightly dilated	1:6,000,000

<sup>1</sup> Omentum exposed by abdominal field block with 1% procaine. <sup>2</sup> Topical application of epinephrine—minimal effective concentration.

### Hemorrhagic Shock

*Morphine prior to blood infusion.* A series of 7 dogs were bled to shock levels and morphine was administered during different stages of shock (table 2). During the hyperreactive stage, morphine had no vasodepressor action (*dog 154*—local procaine anesthesia, and *dog 36*—morphine sulfate—12 mg/kg.). One animal (*dog 153*—cyclo-propane) was given morphine during the transitional stage just before the peripheral blood vessels became hyporeactive. The blood pressure fell slightly for a short period and then returned to its original level. Four dogs (*nos. 100, 101*—morphine sulfate—6 mg/kg.; *no. 23*—morphine sulfate—2 mg/kg.; *no. 146*—no general anesthesia) were given morphine during the hyporeactive stage of shock and in 3 of these (*dogs 100, 101, 146*) the blood pressure fell precipitously, necessitating immediate transfusion.

*Morphine following infusion of blood.* Eleven dogs anesthetized with morphine sulfate (2 mg. intravenously or 12 mg. subcutaneously/kg. body weight) and two dogs receiving no general anesthesia were bled by graded hemorrhage until the vascular bed in the omentum had become hyporeactive (in two of the dogs, the hyporeactive stage was determined by assay of the blood). The blood pressure in these animals was on the average from 30 to 40 mm. Hg. The reactivity of the terminal arterioles to epinephrine was in the range of one part in 500,000 as compared with con-

trol responses to one part in 8 to 10,000,000 of epinephrine. Vasomotion was depressed and often absent. Blood flow through the collecting venules and the larger veins (100-150  $\mu$ .<sup>1</sup>) was sluggish. The animals were then infused with all of the blood previously withdrawn. The blood pressure in all cases but one (*dog 99*) returned to the 100 to 120 mm. Hg range. With the restored blood volume, mechanical speeding up of blood flow through the capillary bed occurred. The terminal arterioles underwent a moderate narrowing. The responsiveness of the muscular vessels to epinephrine rose, but did not regain control values. Vasomotion was only slightly influenced, if at all, usually remaining depressed.

TABLE 2. MORPHINE ADMINISTERED DURING SHOCKED STATE

DOG	ANESTH.	STAGE OF SHOCK		MOR- PHINE DOSE <sup>1</sup> <i>mg/kg.</i>	EFFECTS		REMARKS
		B.P. X min.	Omentum		Blood Pressure	Omentum	
154	None	<80 mm. X 30	Hyper-reactive	2	No change	Unaffected	Recovered spontaneously
36	M.S. <sup>2</sup>	<60 mm. X 120	Hyper-reactive	3	No change	Unaffected	Reversible by infusion
153	Cyclop.	<80 mm. X 90	Transitional	2	Transient fall to 60 mm.	Flow slowed	Reversible by infusion
100	M.S.	<40 mm. X 90	Hypore-active	3	Fell to 25 mm.	Stagnant	Required infusion to halt circulatory depression
101	M.S.	<40 mm. X 90		3	Fell to 20 mm.		Died despite infusion of blood
23	M.S.	<60 mm. X 120	Hypore-active	1	Fell to 40 mm.	Slowed, arterioles dilated	Irreversible to infusion
146	M.S.	<60 mm. X 180	Hypore-active	2	Fell to <20 mm.	Stagnant	Died in 3 hours despite large blood infusion

<sup>1</sup> Intravenously administered.   <sup>2</sup> M.S. = morphine sulfate.

At this stage, morphine sulfate was given to these animals. In all but two cases (table 3, *dogs 19, 72*), in contrast to the absence in unbled animals of any observed effect of such doses of the drug, the blood pressure fell to subnormal levels (a drop of 40-50 mm. Hg) within 3 to 15 minutes. It then continued to drop more slowly from these values. Concomitant with the fall in blood pressure, the arterioles and pre-capillaries in the omentum became dilated. The blood flow through the capillary bed, especially on the venous side, became sluggish. The threshold response of the muscular vessels to epinephrine rapidly fell to the depressed values present during the hyporeactive stage of shock. Only one of the affected animals (*dog 68*) showed a spontaneous recovery from this condition, returning to a blood pressure of 85 mm. Hg within 45 minutes. In the remaining 9 cases the hypotension persisted and it was found necessary to transfuse the dogs in order to avoid a rapid exitus. Four of the dogs died despite repeated transfusion therapy at this stage.

## DISCUSSION

It has been shown previously that morphinized dogs (2–6 mg/kg.) developed a more pronounced depression of the peripheral vascular system in the terminal stage of hemorrhagic shock than non-morphinized control animals (7). However, no major

TABLE 3. MORPHINE ADMINISTERED TO SHOCKED DOGS AFTER BLOOD INFUSION DURING HYPOREACTIVE STAGE

DOG	INITIAL DOSE M.S. <sup>1</sup>	CONDITION OF ANIMAL				MORPHINE DOSE <sup>2</sup>	EFFECTS		REMARKS	
		Pre-infusion <sup>3</sup> Blood Pressure	Post-infusion		Blood pressure	Omentum				
			Blood pressure	Omentum		Blood Pressure	Omentum			
		per kg.	mm. Hg X hrs.	mm. Hg X hrs.		mg./kg.	mm. Hg			
19	2 mg.	<70 X 3.0	110 X 3.0	Normal reactivity	0.4	No change	No change		Animal reversible	
68	12 mg.	<70 X 4.5	100 X 0.5	Normal reactivity	1.0	Fell to 75	Slowing of flow		B.P. and flow restored in 25 min.	
111	None	<40 X 2.5	110 X 0.5	Arterioles tonic	2.0	Fell to 70	Flow stagnant		B.P. and flow restored only by infusion of 150 cc. blood	
107	None	<40 X 1.5	100 X 0.1	Hyporeactive	2.0	Fell to 60	Flow sluggish		Died in 20 min., despite infusion	
99	2 mg.	<50 X 4.0	50 X 0.5	Hyporeactive	2.0	Fell below 20	Complete cessation of flow		Died in 10 min. despite infusion	
38	12 mg.	<60 X 4.5	90 X 0.75	Reactivity somewhat improved	2.7	Fell to 70	Flow slowed especially in veins		Lived 3 hrs. later	
56	12 mg.	<60 X 3.5	100 X 0.5	Hyporeactive	3.6	Fell to 70	Dilatation of arterioles		B.P. and flow restored by infusion of saline 10 cc/kg.	
47	12 mg.	<50 X 3.0	100 X 1.0	Tone of vessels somewhat improved	4.2	Fell to 80	Pronounced slowing of flow		B.P. and flow slowly restored by repeated infusions	
78	12 mg.	<60 X 6.0	110 X 2.0		4.8	Slight fall			No change for 1 hr. then sudden collapse	
57	12 mg.	<50 X 3.0	115 X 0.5	Circulation improved, reactivity still sub-normal	6.0	Fell to 70	Temporary slowing of flow		B.P. and flow restored by saline-pitressin infusion	
30	12 mg.	<70 X 5.5	100 X 2.0	Circulation almost normal	6.0	Fell to 60	Arterioles dilated		B.P. and flow restored by infusion of saline 5 cc/kg.	
58	12 mg.	<50 X 5.0	105 X 0.5	Circulation improved, reactivity still sub-normal	9.0	Fell to 30	Stagnant		B.P. and flow restored by saline-albumin infusion 6 cc/kg.	
33	12 mg.	<50 X 4.0	100 X 0.2		10.0	Fell to 65			Died in 1.5 hrs. despite infusion	

<sup>1</sup> M.S. = morphine sulfate.

<sup>2</sup> Omentum in hyporeactive state.

<sup>3</sup> Intravenously administered.

differences were observed in the depth or duration of the hypotension, or in the relative blood-loss necessary to produce eventual irreversibility to whole blood restoration. Similarly, both in dogs with no general anesthesia and dogs under morphine, the hyperreactive phase of early hemorrhage was of the same general range and duration. These findings, together with the comparatively unimpaired vascular reactivity found in the morphinized unbled dogs in this study, suggest that the periph-

eral vasomotor mechanisms prior to and during early blood-loss can compensate for any vasodepressor properties of morphine in doses of 1 to 3 mg/kg.

The effects of morphine during the subsequent course of the hemorrhagic shock syndrome appear to depend upon the duration of profound hypotension and the dose of morphine employed. Thus, the three animals receiving 2 to 3 mg. of morphine per kg. of body weight prior to the development of hyporeactivity in the omentum showed either a negligible or transient fall in blood pressure and slowing of omental circulation. This is in agreement with the observations of Reed and co-workers who likewise administered morphine early in hemorrhagic hypotension (3).

Morphine administered during the hyporeactive stage of shock was followed by a profound and sustained fall in blood pressure together with a considerable slowing of the peripheral circulation. Morphine administered to dogs which had been transfused during the hyporeactive stage of shock likewise produced a rapid deterioration of the peripheral circulation and a significant lowering of the blood pressure. It is evident that animals which have already suffered peripheral vascular damage are especially susceptible to the vasodepressor action of morphine. This is of considerable significance in the use of morphine as an analgesic agent in the immediate post-hypotensive period. In dogs subjected to hemorrhage such medication is definitely contraindicated.

The precise mechanism whereby morphine acts to depress the peripheral circulation is not clear. The presence of considerable amounts of a vasodepressor principle VDM in the blood during the latter stages of hemorrhagic shock suggests that morphine may intensify the decompensatory action of this principle on the peripheral vessels. Whether the effect is myotoxic or is mediated through an interference with the sympathetic vasoconstrictor impulses in the terminal vessels remains to be determined.

#### SUMMARY

Based on changes in blood pressure and direct visual observation of omental peripheral blood vessels, usual doses of morphine (2-12 mg/kg.) in unhemorrhaged dogs produce no notable disturbances.

Morphine (2-3 mg/kg.), when given early in hemorrhagic shock, is followed by no sustained vascular depression. Morphine (0.4 to 10 mg/kg.) administered during latter stages of hemorrhagic shock usually lowers the blood pressure and depresses the peripheral circulation for a variable period of time. The vasodepressor effect does not appear to be directly related to the dosage administered.

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# EFFECT OF ALTITUDE ON RESPIRATORY FLOW PATTERNS<sup>1</sup>

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**S**TUDIES of breath velocity and the timing of the various phases of the breath were begun by us in connection with the physiological assessment of high altitude oxygen equipment.<sup>2</sup> The need for fundamental data regarding the behavior of breathing in response to physical changes in the atmosphere is apparent from the lack of treatment of this subject in physiology texts and the preoccupation of physiologists with the effects of variations in the partial pressure of oxygen and carbon dioxide.

Fleisch (1, 2) and Bretschger (3) made breath velocity analyses, demonstrating some of the neuromuscular and physical factors which interact in pulmonary ventilation. Gukelberger (4, 5) made particularly sensitive analyses of normal breath velocity patterns in terms of acceleration values, but without attempting physical analysis of the non physiological components. Silverman *et al.* (6, 7) studied the effects of various pulmonary disease conditions on the breath velocity pattern but did not consider the methods of pattern analysis which had been made by Gukelberger, probably because of the isolation of European literature occasioned by World War II. Silverman's analysis did not consider physical changes in the inspired air as used in palliative and therapeutic helium treatment. Dean and Visscher (8), on the other hand, did not use the pneumotachographic method in analyzing the mechanism of helium oxygen administration in pulmonary obstruction therapy. Thus, despite a few observations by Pappenheimer and Lilly (9) in 1943 and the 1926 work of Fleisch (2) on breath mechanics at altitude (which, incidentally, demonstrated certain of the phenomena reported here and indirectly supports our hypotheses) the essential effects on breathing of the physical factors introduced by the atmosphere, have not been recognized.

While our studies originated in response to the need for flow patterns which would facilitate the efficient and safe design of oxygen regulators and mask valves, the studies soon led into considerations of the relations between various phases of the breath and their bearing on the efficiency of pulmonary functions affecting gaseous exchange in the pulmonary system. The data presented are intended to show primarily the norms of breathing behavior under rather special conditions, and secondarily, the physical basis for the responses of the pulmonary ventilation to breathing at high altitude, and their possible application in assays of pulmonary function.

Early observations (10) on respiratory behavior made with closed circuit oxygen

Received for publication January 11, 1949.

<sup>1</sup>A resume of this work was presented at the Symposium on Military Physiology Dec. 4-6, 1947, at Walter Reed Hospital, Washington, D. C.

<sup>2</sup>The Engineering Division, Bureau of Aeronautics, Navy Department, sponsored this work under T.E.D. No. NIH 2517. The Bureau of Medicine and Surgery, Research Division, Navy Department encouraged the prosecution of these studies in respiratory phenomena, and we are indebted to the Naval Medical Research Institute for furnishing us with medically screened volunteers from their staff.

regenerating systems indicated to us that ascent to 30,000 feet simulated altitude induced a decrease in respiratory rate and a compensatory increase in tidal volume resulting in relative constancy of minute volume. It is probable that the attainment of this relation was fortuitous and due to the particular experimental setup, but the general effect of altered breath timing has been substantiated in our later work. Since full oxygenation of the blood was assured at altitude by the conditions of the experiments, it was deduced that, in contradiction to all but Barach's (11) hypothesis, the factor which brought about these changes was gas density or a related physical



Fig. 1. FLOW MEASURING INSTRUMENT AND NECESSARY APPARATUS: *a*, wire suspension; *b*, breathing tube; *c*<sub>2</sub> and *c*<sub>3</sub>, bracing; *d*, valve housing; *e*, expiratory tube; *f*, inspiratory tube with outlet; *g*, spirometer; *h*, iris diaphragm resistance on inspiratory tube; *i*, mercury arc lamp; *j*, projection system to camera (not shown); *k*, heater unit to prevent condensation on window; *l*, pressure tube to *m*, relay switch for activating *n*, spring opposed solenoid; *o*, Borda mouthpiece to replace valve when breathing air.

property. On the strength of these observations a series of experiments was set up to measure quantitatively the changes induced in the timing of the phases of the breath cycle and in breath velocities.

#### APPARATUS AND TEST CONDITIONS

The desirability of influencing breath velocity as little as possible by frictional resistance in the air stream (which would also respond to changes in density) led to the adaptation of the flowmeter design used by Silverman (12) modified in certain respects as described in (13) and shown in figure 1. Essentially, the instrument

records photographically the deflection of the shadow of a fine wire stretched through the breath stream. Since a record of both expiratory and inspiratory flows was desired, it was necessary to add dead space in excess of that of the mask. Data presented by Stannard and Russ (14) on the effects of added dead space on the ventilation indicate that a gain of about 16 per cent in the tidal volume results from a dead space of about 150 cc. (slightly more than the volume of the flowmeter). They found no consistent influence on the breathing rate.

A double butterfly valve (13) actuated indirectly by the breath pressure was used to insure proper circulation of the respired gases from the distal end of the flowmeter. It offered minimal resistance to all flows encountered. A backflow, of low order as compared to sensitive rubber valves (12) used for the same purposes, was incurred by the unavoidable delay of the tripping mechanism. In common with other valves, efficiency rises with the respiratory rate and breath velocity.

The valve openings were connected distally to a closed oxygen circuit of 1.25 inch i.d. rubber tubing, disposed in such a manner as to avoid sharp turns and connecting with a recording spirometer which had a CO<sub>2</sub> absorbing filter in the expiratory duct and an adjustable iris diaphragm for adjustment of resistance in the inspiratory duct.

Measurements of the pressure drop from mask to spirometer with constant velocity, using a rotameter in place of the subject, were made with a slant manometer. The pressure drops at various airflows were as follows:

L/MIN.	'EXPIRATION' PRESSURE IN MM. H <sub>2</sub> O	'INSPIRATION' SUCTION IN MM. H <sub>2</sub> O
20	= 1.4	= 1.1
40	= 2.2	
50	= 3.9	= 2.0
70	= 5.8	= 3.0
80	= 7.5	
100	= 9.0	= 4.0

Measurements with oxygen instead of air gave essentially equivalent values, indicating that orifices in this system were large enough to have only a negligible influence at the velocities encountered.

The restriction caused by a standard setting of the iris diaphragm resulted in a 100-fold increase in inspiratory suction at peak flows, as shown by the following data, which suggest a slight difference between air and oxygen in this respect:

L/MIN.	'INSPIRATION' (RESTRICTED) AIR SUCTION IN MM. H <sub>2</sub> O	'INSPIRATION' (RESTRICTED) OXYGEN SUCTION IN MM. H <sub>2</sub> O
20	= 15	= 20
30	= 38	= 45
40	= 83	
50	= 140	= 154
60	= 212	= 227
70	= 303	= 318

A comparison of measurements of breath patterns obtained when breathing air through the flowmeter alone and through the flowmeter with the added valve and spirometer circuit were made in order to assess the alterations induced by these

factors of resistance. As indicated above, the flow pressures due to unrestricted flows of the order encountered in normal breathing (up to 70 l/min.) were very low. Such pressure changes are minor in comparison with other uncontrolled factors affecting breath velocity, and hence do not correlate with respiratory responses. However, paired experiments on five subjects showed that the addition of the valve and spirometer caused a statistically significant 31 per cent increase in the length of the respiratory cycle; a 10 per cent decrease in the maximum velocity of inspiration and a 32 per cent increased rate of termination of inspiration ( $I_{pd}$ ) were not statistically significant.

It was fully appreciated that so-called 'normal' conditions of breathing could not be expected, even though further precautions might be taken, since most individuals change their breathing response when confronted with a mask and respiratory measuring devices. It is felt that a knowledge of these changes induced by the experimental procedure will assist in the interpretation of the data.

#### SUBJECTS AND TEST PROCEDURE

Subjects for this study were 32 male volunteers, previously indoctrinated for altitude work by the Naval Medical Research Institute staff. Their ages were: 6 at 17 years; 20 at 18 years; 1 at 19 years; 1 at 26 years; 1 at 29 years; 2 at 30 years, and 1 at 36 years. In general, they were not apprehensive, since the whole test routine was run off at ground level before the altitude run was made. A few experienced some difficulty on descent due to ear block, but this was subsequent to the tests. Only one man reported gas pains at altitude but was able to complete the tests.

A concise outline of procedure in non-technical words was given before the test in order to obtain proper cooperation in maintaining the seal of the mask and in following the variations of the procedure. It was emphasized that the subject was breathing full oxygen at all times. The breathing circuit was flushed out with  $O_2$  before each recording.

The subjects sat with chest erect so that the face rested firmly in the inflated rim of the mask. The principal opening of the mask was of the same bore as the flowmeter and was placed directly before the mouth and nares. The subject was instructed to breathe as naturally as possible both in the trial period and during the recording. After the test was completed it was ascertained whether or not the subject had resorted to mouth breathing.

The record was preceded by a baseline recording which required the subject to hold his breath momentarily, and this was followed by a series of 8 to 10 breaths before further recording. At the end of this interval, inspection of the spirogram indicated to the operator whether or not a characteristic record could be taken.

A record of at least 4 consecutive breaths was taken with the subject at rest. This was followed by a record during an arbitrary constriction of the inspiratory path by means of the iris diaphragm. The subject was then exercised by making 15 deep knee bends from a standing position at a rate of his own choosing, but not including perceptible pauses. The subject steadied himself, if necessary, by one hand.

Following the exercise the subject breathed through the flowmeter, and a record was made without intervening breath holding in order to obtain the most enhanced velocities subsequent to the exercise. A second bout of exercise, but limited to 10 deep knee bends, was performed by the subject prior to breathing on the restricted circuit. Pulse rates were recorded after both bouts of exercise to indicate whether or not equivalent stress had been effected. The pulse rates approximated 125 to 130/minute and indicate a work equivalent to about 600 kg.M./min. (5).

This routine was repeated precisely shortly after attaining an altitude of 30,000 feet at a rate of 3000 feet per minute. The subject was provided with full oxygen



Fig. 2. ONE RESPIRATION from sample tracing showing most of the points discussed. Subject at rest breathing O<sub>2</sub> at 30,000 ft. simulated altitude. Time signals at bottom of record in seconds; horizontal lines 5 mm. apart. Ink spots show approximate location of numbered reference points: 1 to 2 = duration of inspiration (*Is*); 2 to 3 = duration of expiration (*Es*); 3 to 4 = duration of expiratory pause (*Eps*); 1 to 4 = duration of total breath (*Bs*); 5 = inspiratory maximum deflection (87 l/min.) (*Imx*); 8 = expiratory maximum deflection (132 liters per minute) (*Emx*); 1 to 5 = inspiratory acceleration (*Ia*); 2 to 7 = expiratory acceleration (*Ea*); 5 to 6 = inspiratory plateau (*Ip*); 7 to 8 = expiratory plateau (*Ep*); 6 to 2 = inspiratory drop (*Id*); 8 to 3 = expiratory drop (*Ed*).

during ascent and at altitude, and changed from mask to flowmeter and back while holding the breath momentarily.

Velocity calibrations were made after each run and applied to the individual records.

#### METHOD OF ANALYSIS OF THE DATA

The primary data obtained from these tests were the breathing pattern photographically registered from the wire shadow and the displacements of the spirometer in the breathing circuit. A sample tracing is shown in figure 2.

The velocity pattern was resolved into the following items which appeared to be phenomena common to each breath, except as noted:

1. *Inhalation.* The time in seconds from the rise from the resting position of the wire to the return to that level, (*Is*).

2. *Inspiratory-expiratory pause.* The time in seconds from end of 1 to the crossing of the wire in the opposite direction from the resting position, (Ips). Absent in most individuals, especially after exercise.

3. *Expiration.* The time in seconds from the end of 2 to the return of the wire to the resting position, (Es).

4. *Expiratory-inspiratory pause.* The time in seconds from 3 to the rise from the resting position of the wire denoting the next inspiration, (Eps). Many individuals do not have it at ground level.

5. *Total time for one breath cycle in seconds,* (Bs).

6. *Rate of respiration,* (60/Bs).

7. *Inspiratory 'peak' velocity.* The velocity attained at the beginning of the plateau phase in inspiration in liters per minute (Imf).

8. *Expiratory 'peak' velocity.* As in 7 but for the expiratory phase.

Both the inspiratory and expiratory velocity records were further analyzed into three parts: acceleration, plateau and deceleration, as listed below. In our experience the plateau was almost always somewhat rounded, and in approximately three fourths of the cases the plateau had a definite negative slope. The remaining cases were level or had a positive slope.

9. *Inspiratory acceleration.* Rate of increase in velocity in liters/min/sec., (Ia). In tests with inspiratory restriction this was used only in calculating breath volume, since an initial backflow allowed a momentary 'spike' to develop.

10. *Inspiratory plateau.* Magnitude and sign of slope of plateau in liters/min/sec., (Ip).

11. *Inspiratory deceleration.* Rate of decrease in velocity in liters/min/sec., (Id).

12. *Expiratory acceleration.* As in 9 (Ea).

13. *Expiratory plateau.* As in 10 (Ep).

14. *Expiratory deceleration.* As in 11 (Ed).

From the spirometer tracings the following additional measurements were made:

15. *Tidal volume in liters,* (Tv).

16. *Minute volume.* Liters of gas ventilated through the lungs. Calculated from 15 and 6 (Mv).

The photographic records were provided with time lines at 1/240 and 1-second intervals. It was customary to measure time intervals from the record by means of a proportionally adjustable rule in order to allow paper speed differences to be equated. The rule was marked in tenths of a second and allowed estimation of .05 second. The records were run at such a rate as to allow reasonably accurate measurements to be made with this method.

Due to mechanical limitations of stretched or suspended wires, as in this flowmeter, the zero positions following inspiration and expiration were different. The damping of this particular instrument caused the variations in absolute position to be more pronounced than would be the case if free oscillation had been permitted. However, this deviation was known and accounted for in estimating pauses, which appear in the record as level stretches in the pattern in the immediate vicinity of the arbitrary zero position. The maximum zero displacement found in our instrument was  $\pm 3$  mm. from the resting position at an optical magnification of about 250 diameters. This corresponds to an apparent velocity of about 12 liters per minute,

TABLE I. RESPIRATORY PATTERN DATA AT GROUND LEVEL AND ALTITUDE, WITH  
AND WITHOUT EXERCISE

	REST <sup>1</sup>		EXERCISE <sup>2</sup>	
	Ground Level		30,000 Feet	
	seconds	seconds	seconds	seconds
Inspiration	1.430±.298	1.440±.291	1.298±.252	1.293±.294
Insp. pause	.045±.089	.072±.095	.002±.009	.087±.093
Expiration	1.622±.418	1.490±.373	1.333±.262	1.363±.392
Exp. pause	.122±.166	.320±.241	.023±.067	.088±.114
Total	3.217±.762	3.285±.667	2.655±.510	2.831±.793
Respirations per minute	no. 19.69±4.13 (11.3-27.9)	no. 19.27±4.38 (11.8-30.3)	no. 23.48±5.12 (18.3-34.7)	no. 22.83±6.51 (14.0-35.3)
Inspiratory peak flow	l/min. 43.0±9.5 (21-66)	l/min. 43.0±12.4 (12-97)	l/min. 47.9±11.1 (32-74)	l/min. 59.6±27.7 (18-117)
Expiratory peak flow	48.5±12.7 (19-71)	56.9±19.9 (17-134)	54.5±21.8 (16-108)	80.8±41.9 (18-203)
Inspiratory Acceleration	liters/min/sec. 112.6±32.9	121.9±52.7	138.1±49.6	171.8±233.6
Inspiratory Plateau	-12.6±17.0	-16.5±20.2	-12.9±17.6	-6.0±25.2
Inspiratory Deceleration	-98.5±37.1	-106.4±46.1	-126.8±46.2	-197.5±149.1
Expiratory Acceleration	105.4±34.4	132.7±44.6	145.8±65.7	279.3±201.0
Expiratory Plateau	-24.0±26.6	-32.3±32.0	-37.1±22.2	-48.1±60.4
Expiratory Deceleration	-65.9±31.6	-74.9±52.9	-95.2±28.7	-193.6±210.9
Tidal Volume	liters 0.773±0.156 (.52-1.13)	liters 0.696±0.136 (.49-1.10)	liters 0.923±0.304 (.50-1.76)	liters 1.044±0.222 (.66-1.43)
Minute Volume	liters 14.86±3.29 (9.6-25.8)	liters 13.04±2.37 (9.2-19.5)	liters 20.49±4.76 (14.4-28.3)	liters 23.73±9.40 (15.3-50.5)

<sup>1</sup> Values are means of 32 subjects' averages, 2 to 6 breaths per subject, and std. dev.    <sup>2</sup> Values are means of 12 subjects' averages, 3 breaths per subject, and std. dev.    Values in parentheses are ranges.    Negative sign denotes deceleration.

but lies in the lower extrapolated portion of the calibration. This action of the wire did not interfere with the accuracy of measurement of the velocities given in these data since due allowance was made for the proper zero positions.

#### RESULTS

The data compiled from the series of tests on Navy volunteers are shown in tables 1 and 2. The means shown are the means of the mean values for individuals,

weighted equally and thus give an indication of the variation in the population which we studied.

TABLE 2. RESPIRATORY PATTERN DATA AT GROUND LEVEL AND ALTITUDE WITH AND WITHOUT EXERCISE, WITH ADDED INSPIRATORY RESISTANCE

	REST		EXERCISE	
	Ground Level	30,000 Feet	Ground Level	30,000 feet
	seconds	seconds	seconds	seconds
Inspiration	1.422±.288	1.405±.304	1.339±.226	1.231±.260
Insp. pause	.040±.080	.081±.084	.004±.014	.038±.064
Expiration	1.448±.287	1.349±.280	1.263±.232	1.298±.387
Exp. pause	.057±.091	.278±.259	.004±.014	.050±.072
Total	2.968±.627	3.114±.734	2.610±.424	2.618±.670
Respirations per minute	no. 21.16±4.87 (15.9-28.3)	no. 20.39±5.28 (13.9-28.7)	no. 23.62±4.26 (18.5-30.8)	no. 24.23±11.19 (14.4-35.9)
Inspiratory peak flow	l/min. 34.6±8.7 (20-66)	l/min. 34.8±12.4 (13-63)	l/min. 44.8±10.7 (26-72)	l/min. 59.1±18.7 (11-94)
Expiratory peak flow	l/min. 44.9±13.7 (26-75)	l/min. 46.5±17.0 (9-85)	l/min. 63.7±25.6 (27-118)	l/min. 85.2±30.4 (25-144)
Inspiratory Acceleration	liters/min./sec. 133.8±17.8	liters/min./sec. 148.9±75.3	liters/min./sec. 210.3±77.8	liters/min./sec. 388.7±685.6
Inspiratory Plateau	-16.8±8.8	-1.0±14.9	-14.1±18.6	-4.9±13.0
Inspiratory Deceleration	-98.8±45.7	-114.4±70.6	-129.1±45.7	-209.8±106.7
Expiratory Acceleration	108.3±49.7	154.0±55.9	167.8±79.9	299.8±209.2
Expiratory Plateau	-22.6±29.9	39.8±24.0	-40.1±28.0	-70.6±52.7
Expiratory Deceleration	-79.4±37.4	-71.2±64.3	-119.0±79.9	-259.6±224.0
Tidal Volume	liters 0.766±0.133 (0.50-0.94)	liters 0.709±0.171 (0.43-0.97)	liters 0.931±0.219 (0.56-1.34)	liters 1.059±0.257 (0.77-1.44)
Minute Volume	liters 15.92±3.96 (12.3-26.1)	liters 14.11±3.43 (7.0-19.8)	liters 21.47±4.06 (16.1-29.7)	liters 25.55±8.15 (10.4-38.7)

Values are means of 12 subjects' averages, 3 breaths per subject, and std. dev. Values in parentheses are ranges. Negative sign denotes deceleration.

Figure 3 presents graphically the salient points of the tables in order to show the inter-relation of the data for any one condition and also to provide a ready comparison of the effects of altering the several conditions of altitude and exercise. It

must be recognized that the flow patterns as shown in figure 3 are derived in all instances from rounded curves as in figure 2. It is felt that the beginning of inspiration is the logical point of origin for these diagrams, and this presentation tends to distinguish curves having even small timing differences better than would the use of the inspiratory-expiratory crossover point as the point of coincidence of the several types of pattern (15).

#### DISCUSSION

The following statistical treatment of the data is made on the basis of paired values from each subject. The mean of the differences for the various subjects is

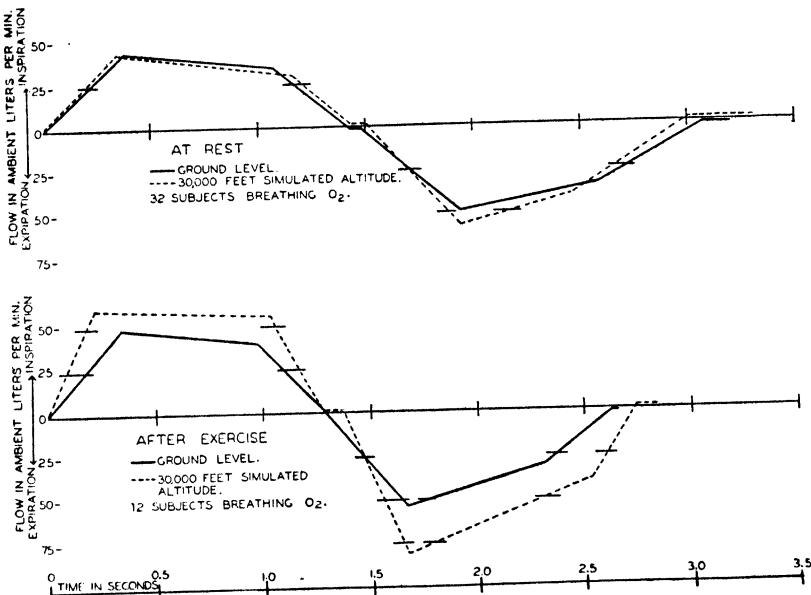


Fig. 3. BREATHING PATTERNS at ground level and at altitude.

given below, accompanied by a statistical evaluation in terms of standard deviation of the differences, standard deviation of the mean, and the probability of the difference being due to chance, and the ratio of the difference to the control mean in percentage. Comparisons showing no statistical significance for the difference are not given since they may be obtained from the tables.

*Effect of Altitude on Respiratory Pattern.* In the resting condition without added inspiratory resistance, ascent to 30,000 feet alters:

$$\begin{aligned}
 Eps, +.20 \text{ sec.}, s = .24, \bar{s} = .04, P = .001, +167\% \\
 Emf, +8.371/\text{min.}, s = 15.30, \bar{s} = 2.70, P = .001 \text{ to } .01, +17.2\% \\
 Ea, +27.251/\text{min./sec.}, s = 36.46, \bar{s} = 6.45, P = .001, +25.9\% \\
 Tv, -.078 \text{ liters}, s = .110, \bar{s} = .019, P = .001, -9.9\% \\
 Mv, -.181 \text{ liters}, s = 2.23, \bar{s} = .39, P = .001, -12.1\%
 \end{aligned}$$

When restriction is added to the inspiratory path at rest, as above, ascent to 30,000 feet alters:

$$\begin{aligned} \text{Eps, } +.222 \text{ sec., } s = .255, \bar{s} = .074, P = .01, +369\% \\ \text{Ip, } +15.81/\text{min./sec., } s = 12.9, \bar{s} = 3.7, P = .001 \text{ to } .01, +92.8\% \\ \text{Ea, } +45.61/\text{min./sec., } s = 68.3, \bar{s} = 19.7, P = .02 \text{ to } .05, +42.2\% \end{aligned}$$

Following exercise, without added restriction, ascent to 30,000 feet alters:

$$\begin{aligned} \text{Ips, } +.084 \text{ sec., } s = .082, \bar{s} = .024, P = .001 \text{ to } .01 \\ \text{Emf, } +26.3 \text{ l/min., } s = 35.9, \bar{s} = 10.4, P = .02 \text{ to } .05, +47.8\% \\ \text{Ia, } +133.71/\text{min./sec., } s = 195.3, \bar{s} = 56.4, P = .02 \text{ to } .05, +96.8\% \\ \text{Ea, } +133.41/\text{min./sec., } s = 178.6, \bar{s} = 51.5, P = .02 \text{ to } .05, +91.3\% \end{aligned}$$

When resistance is added to the inspiratory path after exercise, ascent to 30,000 feet alters:

$$\begin{aligned} \text{Eps, } +.046 \text{ sec., } s = .069, \bar{s} = .020, P = .02 \text{ to } .05 \\ \text{Imf, } +14.31/\text{min., } s = 12.7, \bar{s} = 3.7, P = .001 \text{ to } .01, +31.8\% \\ \text{Emf, } +21.51/\text{min., } s = 21.4, \bar{s} = 6.2, P = .001 \text{ to } .01, +33.6\% \\ \text{Id, } -80.7, \text{l/min./sec., } s = 87.7, \bar{s} = 25.3, P = .001 \text{ to } .01, -62.5\% \\ \text{Ea, } +132.81/\text{min./sec., } s = 186.1, \bar{s} = 53.7, P = .02 \text{ to } .05, +79.0\% \\ \text{Ep, } -30.51/\text{min./sec., } s = 16.5, \bar{s} = 4.8, P = .001, -76.3\% \\ \text{Ed, } -140.61/\text{min./sec., } s = 182.8, \bar{s} = 52.8, P = .02, -117.6\% \\ \text{Tv, } +.128 \text{ liters, } s = .182, \bar{s} = .053, P = .02 \text{ to } .05, +13.7\% \end{aligned}$$

In no case was there a significant change in average inspiratory or expiratory time, although this may be largely due to the rather marked variation in response. It was further observed that the breath interval, Bs, did not change significantly with altitude even though statistically significant changes in the pauses may have occurred. This also may be due largely to the marked variation. In view of this variation the distribution of the data is of interest. The distribution was found to be normal and, in particular, no bimodal tendencies were observed. This eliminates the possibility that a salutation to both sides of the normal value, still maintaining a stable average, might have been induced by the factor under investigation.

Retesting subjects on a subsequent day, both at ground level and at altitude in the resting condition, showed that statistically significant changes, some negative and most of them positive, occurred in Is, Ips, Bs, Emf, Ea, and Tv due to unknown causes. However, no difference was found in control tests repeated within about one hour. It is noteworthy that despite an increase in ambient volume of backflow at altitude, a decrease in both tidal volume and minute volume occurs at rest, and only a moderate increase occurs after exercise.

*Effect of a Standard Exercise on Breathing Pattern.* At ground level, without added resistance, the imposition of exercise alters:

$$\begin{aligned} \text{Es, } -.142 \text{ sec., } s = .150, \bar{s} = .043, P = .001 \text{ to } .01, -9.6\% \\ \text{Bs, } -.358 \text{ sec., } s = .288, \bar{s} = .083, P = .001 \text{ to } .01, -11.9\% \\ \text{Emf, } +10.91/\text{min., } s = 16.5, \bar{s} = 4.8, P = .02 \text{ to } .05, +24.8\% \\ \text{Ep, } -18.21/\text{min./sec., } s = 26.3, \bar{s} = 7.6, P = .02 \text{ to } .05, -95.8\% \\ \text{E.l, } -29.51/\text{min./sec., } s = 29.5, \bar{s} = 8.5, P = .001 \text{ to } .01, -44.6\% \\ \text{Mv, } +4.54 \text{ liters, } s = 3.85, \bar{s} = 1.11, P = .001 \text{ to } .01, +28.4\% \end{aligned}$$

At ground level, with added inspiratory resistance the effect of exercise is to alter:

Es,  $- .185 \text{ sec.}, s = .144, \bar{s} = .042, P = .001, - 12.8\%$   
 Eps,  $- .052 \text{ sec.}, s = .081, \bar{s} = .023, P = .02 \text{ to } .05, - 86.6\%$   
 Bs,  $- .358 \text{ sec.}, s = .312, \bar{s} = .090, P = .001 \text{ to } .01, - 12.1\%$   
 Imf,  $+ 10.3 \text{ l/min.}, s = 9.1, \bar{s} = 2.6, P = .001 \text{ to } .01, + 29.6\%$   
 Emf,  $+ 18.81 \text{ l/min.}, s = 15.6, \bar{s} = 4.5, P = .001 \text{ to } .01, + 41.7\%$   
 Id,  $- 30.3 \text{ l/min./sec.}, s = 32.7, \bar{s} = 9.4, P = .001 \text{ to } .01, - 30.5\%$   
 Ea,  $+ 59.31 \text{ l/min./sec.}, s = 53.7, \bar{s} = 15.5, P = .001 \text{ to } .01, + 54.9\%$   
 Ed,  $- 39.6 \text{ l/min./sec.}, s = 40.5, \bar{s} = 11.7, P = .001 \text{ to } .01, - 50.0\%$   
 Tv,  $+ .165 \text{ liters}, s = 1.66, \bar{s} = .048, P = .001 \text{ to } .01, + 21.4\%$   
 Mv,  $+ 5.55 \text{ liters}, s = 4.60, \bar{s} = 1.33, P = .001 \text{ to } .01 + 34.9\%$

At 30,000 feet, without added resistance, the imposition of exercise alters:

Eps,  $- .197 \text{ sec.}, s = .210, \bar{s} = .061, P = .001 \text{ to } .01, - 70.2\%$   
 Imf,  $+ 21.8 \text{ l/min.}, s = 24.6, \bar{s} = 7.1, P = .01 \text{ to } .02, + 57.3\%$   
 Emf,  $+ 38.7 \text{ l/min.}, s = 40.0, \bar{s} = 11.5, P = .001 \text{ to } .01, + 92.2\%$   
 Ea,  $+ 133.61 \text{ l/min/sec.}, s = 197.3, \bar{s} = 57.0, P = .02 \text{ to } .05, + 91.5\%$   
 Tv,  $+ .329 \text{ liters}, s = .176, \bar{s} = .051, P = .001, + 45.7\%$   
 Mv,  $+ 9.94 \text{ liters}, s = 9.33, \bar{s} = 2.69, P = .001 \text{ to } .01, + 68.0\%$

Restriction, under conditions of exercise, at 30,000 feet alters:

Is,  $- .174 \text{ sec.}, s = .246, \bar{s} = .071, P = .02 \text{ to } .05, - 12.4\%$   
 Eps,  $- .228 \text{ sec.}, s = .231, \bar{s} = .067, P = .001 \text{ to } .01, - 81.3\%$   
 Bs,  $- .497 \text{ sec.}, s = .506, \bar{s} = .146, P = .001 \text{ to } .01, - 16.0\%$   
 Imf,  $+ 24.3 \text{ l/min.}, s = 14.7, \bar{s} = 4.3, P = .001, + 69.7\%$   
 Emf,  $+ 38.7 \text{ l/min.}, s = 20.6, \bar{s} = 5.9, P = .001, + 82.3\%$   
 Id,  $- 95.3 \text{ l/min./sec.}, s = 83.1, \bar{s} = 24.0, P = .001 \text{ to } .01, - 83.5\%$   
 Ea,  $+ 145.8 \text{ l/min./sec.}, s = 196.8, \bar{s} = 56.8, P = .02 \text{ to } .05, + 94.6\%$   
 Ed,  $- 188.4 \text{ l/min./sec.}, s = 198.1, \bar{s} = 57.2, P = .001 \text{ to } .01, - 265.3\%$   
 Tv,  $+ .350 \text{ liters}, s = .129, \bar{s} = .037, P = .001, + 49.3\%$   
 Mv,  $+ 11.44 \text{ liters}, s = 6.49, \bar{s} = 1.87, P = .001, + 81.0\%$

These analyses show that the most consistent changes produced by exercise are in the maximum expiratory velocity (Emf) and in the minute-volume (Mv). The only categories in which no significant change is effected in any of the several conditions are the inspiratory pause (Ips) and the inspiratory plateau (Ip). The former being an infrequent phenomenon at best makes this consideration of doubtful significance.

*Effect of Restriction of Inspiratory Path on Breathing Pattern.* In general restriction doubles the number of categories of significant changes brought about by exercise, and enhances the effect in nearly every category. It similarly can be seen to increase the number of categories of significant changes produced by altitude under conditions of exercise, although the nature of the changes varies between individuals. Difficulties in technique introduced by restriction in the inspiratory line themselves preclude a more rigorous analysis.

*General.* In view of the uncontrollable factors of motivation and other psycho-motor effects incident to the mechanics of the test and chamber operation, repeated tests were not made routinely during the same day. In retrospect, this seems to constitute an omission in the data which might have furnished a clue to effects of the

disturbing factors mentioned above, but in view of the added number of controls demanded and the mass of observations necessary to assess such psychomotor states it was felt that this limitation in procedure was justifiable.

Measurement of the areas delineated by the graphs shown in figure 3 indicates that, in the normal resting condition, both at ground level and at altitude, a greater volume is exhaled than inhaled. The difference is of the order of 13 and 17 per cent, respectively, and appears to be real, even though simplification of the pattern has resulted in arbitrarily reducing the areas from those delineated by the rounded curves of the control records (fig. 2). This apparent paradox may be accounted for on the hypothesis that increases in the volume of inhaled gases caused by heat and water vapor, outweigh the loss of volume due to the respiratory exchange of oxygen and carbon dioxide. Bretschger's (3) data show the reverse relation to hold in his measurements using a pressure drop method of flow measurement. The constant loss of inert gas from the body while breathing with oxygen apparatus probably does not contribute significantly to this effect. Following exercise at ground level no differences in inhaled and exhaled volumes were apparent. This follows, in principle, from considerations of the limitation in effectiveness of the factors listed above, especially since pauses are reduced or eliminated. At altitude, on the other hand, records of breathing after exercise again show a difference between inspired and expired gas volumes concomitant with the interposition of pauses. This phenomenon emphasizes the fact that the flowmeter measures the actual velocity of the medium presented to it under ambient conditions, and thus yields a faithful datum for consideration in the design of breathing equipment.

The breath patterns of a given individual look as unique as fingerprints, as noted by Fleisch (1), Bretschger (3), Gukelberger (4, 5) and others. A method of characterizing individual patterns quantitatively is not yet available, but may be feasible with a detailed statistical analysis of individual features of the breath pattern such as made in this series of tests.

From tables 1 and 2 it is seen that, with full oxygenation and submaximal dead space, the distribution of the data on the breathing pattern is probably influenced by the interaction of a group of factors, only a few of which were controlled in these tests, and many of which may well be individual variables. No attempt has been made here to sort the individual patterns into characteristic groups, although this is contemplated on a larger group of subjects.

The data indicate that the effect of altitude on the breathing mechanism is greatest on the expiratory phase, although it affects nearly all features of the act. Fleisch (2) reported, in this connection, that hypoxic subjects at altitude (up to 23,000 feet) introduced post-expiratory pauses despite the hyperventilation incurred by breathing ambient air. These did not occur in the illustration given by Pappenheimer and Lilly for 44,000 feet (9). It appears that ventilation in a rarefied atmosphere at adequate oxygen pressure is carried out so expeditiously via the proprioceptor controlled mechanisms that the chemical initiation of inspiration by CO<sub>2</sub>, which is governed normally by metabolic conditions, appears to lag more than at ground level. This deduction leads to the hypothesis that individuals vary in their ability to supply their bodies with oxygen and remove carbon dioxide via

the lungs, and that this is correlated with the degree to which they use interphasic pauses in their breath cycle. It appears possible to identify individuals with barely adequate ventilatory capacities by reducing or increasing the density of the respired gases, by simulating altitude, diluting with helium or krypton and noting the density level at which interphasic pauses appear. Substantiation of the effect of density on breath velocity by use of helium-oxygen mixtures has been carried out on a different group of volunteers.

We wish to acknowledge the unstinted technical assistance of H. F. Brubach, N. Smith, P. D. Altland and F. Smith and to thank them as well as W. F. Bowen and W. S. Baum for acting as subjects in preliminary trials of the recording equipment. We are indebted to M. Zelle for guidance in statistical treatment of the data.

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# A COMPARISON OF THE RESPIRATORY ACTIVITY AND HISTOLOGICAL CHANGES IN ISOLATED PANCREATIC TISSUE

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**D**EUTSCH AND RAPER (1) found that the rate of respiration of isolated pancreatic tissue was increased by secretin and by acetyl choline with eserine. Harper and Mackay (2) studied the effects of secretin, pancreozymin and vagal stimulation upon the zymogen granule content of the cat's pancreas and the enzyme output in the pancreatic juice. They found that secretin administration, which resulted in the secretion of a juice of low enzyme content, did not affect the granule content of the cells. Vagal stimulation, on the other hand, or the administration of pancreozymin each resulted in an increased output of enzymes in the juice and a diminution in the granules in the cells.

The crude preparations of secretin used by Deutsch and Raper in 1936 probably contained pancreozymin. The present observations were made to compare the effects of pancreozymin and of pancreozymin-free secretin with those of acetyl choline and eserine on the respiration of isolated pancreatic tissue, and to correlate the respiratory effects of these agents with their effects on the zymogen granule content of the isolated tissue. In addition a few observations were made on the effects of histamine and gastrin on isolated pancreatic tissue.

In order to determine whether the energy required for secretion of zymogen granules could be supplied by anaerobic reactions, experiments were carried out in the absence of  $O_2$ , but no secretion was observed even when adenosine triphosphate was added (Cf. Lipmann, 3).

## METHODS

The experiments were performed on isolated pieces of cat pancreas. The animals were fasted overnight. In early experiments the cat was anesthetized with ether followed by chloralose. After an hour or two the pancreas was removed, care being taken to avoid touching the tissue with the fingers, which might possibly initiate secretion by contamination with acetyl choline from the skin. In later experiments the animal was killed by a blow on the head and the pancreas was then removed and used at once.

Samples of the tissue were freed from adherent matter, rapidly weighed on a torsion balance, minced with sharp, small and fine-pointed scissors and placed in the main compartment of a Warburg cup. The pieces of minced tissue were small enough to ensure adequate oxygenation *in vitro* (4). The  $O_2$  uptake, the resultant

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Received for publication February 18, 1949.

of  $O_2$  uptake and  $CO_2$  output, and the anaerobic glycolysis were measured with Warburg manometers in the usual ways at 38°C. (4). Conical cups (20-30 ml. capacity), with a center well and either one or two side arms were used. Anaerobiosis was maintained with a small stick of phosphorus, and  $CO_2$  was absorbed by 0.2 ml. 2N-NaOH in the center well. The cups were equilibrated for 20 minutes in the bath, and thereafter measurements of the gaseous exchanges were made every 5 minutes. When the resting rate of respiration had been obtained (usually within 10 min. of equilibration), acetyl choline or other agents were added from the side arms. Readings were continued until the tissue had been in the bath for 60 minutes. The values of the  $Q_{O_2}$  and  $Q_{CO_2}^{N_2}$  were expressed in  $\mu l./mg.$  dry wt./hr.

The physiological salt solution (bicarbonate saline) of Krebs and Henseleit (5), gassed with 5 per cent  $CO_2$  + 95 per cent  $O_2$  or 5 per cent  $CO_2$  + 95 per cent  $N_2$ , was used during measurements of the balance of the  $O_2$  uptake and the  $CO_2$  output, or of the anaerobic glycolysis of cat pancreas, and the phosphate saline of Krebs (6) during experiments in 100 per cent  $O_2$ . The glucose concentration was 0.2 per cent.

For the estimation of the initial dry weight of tissues, control samples of the pancreas were weighed, washed in distilled water and dried overnight at 110°C. This gave the ratio wet wt./dry wt. for the tissue, from which the (initial) dry weight of the samples incubated could be calculated.

Histological observations were made on the pieces of tissue which had been used for the respiratory experiments, and on pieces which had either been placed in the fixative solution immediately after removal from the animal, or had been placed in saline solution in the Warburg apparatus, but not stimulated in any way. The tissues were fixed and stained by Bensley's Neutral Gentian method as described by Harper and Mackay (2).

The histological observations were concerned with the zymogen granule content of the cells. This is expressed in the tables below as ++++ (cells about  $\frac{3}{4}$  filled with granules); ++ (about  $\frac{1}{2}$  filled with granules); + (about  $\frac{1}{4}$  filled with granules); + (less than  $\frac{1}{4}$  filled with granules). Where marked cell damage was observed no interpretation was attempted.

The secretin and pancreozymin were prepared by a modification of the method described by Harper and Raper (7). The secretin preparations were quite free of pancreozymin and the pancreozymin preparations showed only a trace of secretin activity. The gastrin was prepared by adsorption on a bile acid precipitate from a 60 per cent alcohol extract of the antral mucosa, as described by Harper (8). The material was a powerful stimulant of acid gastric secretion in experiments on cats. In most of these experiments it had no effect on the pancreas, but in a few it showed faint traces of pancreozymin activity.

#### RESULTS

Control observations of two types were made: *a*) Pieces of tissue were placed straight in the fixative so that the initial appearance of the gland could be observed. *b*) Pieces of tissue were incubated in saline alone, in order to determine the resting metabolism of the gland and to see whether under these conditions any diminution in

granule content occurred. It was found that the metabolism was constant or fell slightly. There was in no instance an increase above the initial rate. Slight diminution in zymogen granule content was observed in 4 out of 19 experiments. The resting  $Q_{O_2}$  in 69 samples of isolated pancreatic tissue from a total of 16 cats was from -2.0 to -9.8, average -4.27, standard deviation 1.97.

*Acetyl choline with eserine.* The effects of these agents are illustrated in the results of an experiment in table 1. The effects observed were an increase in respiration with a concomitant decrease in zymogen granules. Acetyl choline, final concentration 0.01 mg. per cent with eserine, final concentration 0.01 mg. per cent had no effect on the  $Q_{O_2}$  (2 experiments). When the same drugs were used at a concentration of 0.1 mg. per cent, the  $Q_{O_2}$  was increased (112%–193%; average 140%, 8 experiments). An experiment at a concentration of 1.0 mg. per cent showed 126 per cent of the resting value. In one experiment with a concentration of 10 mg. per cent, an increase to 156 per cent was observed. Experiments in the bicarbonate saline showed that the increased uptake of  $O_2$  following stimulation was concomitant

TABLE I. EFFECTS OF ACETYL CHOLINE WITH ESERINE ON RESPIRATORY ACTIVITY AND HISTOLOGICAL APPEARANCE OF ISOLATED PANCREATIC TISSUE (1 EXPERIMENT)

TREATMENT OF TISSUE	MAXIMUM RESPIRATORY RESPONSE AS COMPARED WITH CONTROL IN SALINE. $\frac{Q_{O_2} \text{ MAX.}}{Q_{O_2} \text{ CONTROL}} \times 100$	ZYMOGEN GRANULE CONTENT OF THE CELLS
Control sample straight in fixative.....		++++
Control sample in saline.....	100	++++
Acetyl choline 0.1 mg. % eserine 0.1 mg. %.....	136	+++
Acetyl choline 1.0 mg. % eserine 1.0 mg. %.....	126	+
Acetyl choline 10.0 mg. % eserine 10.0 mg. %.....	156	+++

with a similar increased output of  $CO_2$ . There were no histological differences observed between tissue incubated in the bicarbonate or in the phosphate salines.

*Effects of pancreozymin and secretin.* The effects of pancreozymin are illustrated by the results in tables 2 and 3. There was, with the addition of pancreozymin, an increase in respiration. The histological changes observed following the addition of pancreozymin were similar to those following acetyl choline, i.e. a migration of the zymogen granules towards the acinar end of the cell took place and a diminution in the amount of granular material in the cell along with a filling of the ducts with stainable material (where previously they had been empty). Secretin promoted no reduction in the zymogen granule content of the cells but an apparent increase in the zymogen granule content was occasionally observed. This was due to a scattering of the zymogen granules in the cell.

*Atropine.* At concentrations of either 0.1 or 1.0 mg. per cent atropine completely prevented the increase in respiration following acetyl choline with eserine, but it had no effect on the increased rate of respiration following pancreozymin (6 experiments).

*Histamine.* Five experiments were carried out with histamine. In one in which 0.1 mg. per cent of histamine was added, no stimulation of respiration or alter-

TABLE 2. EFFECTS OF PANCREOZYMIN AND SECRETIN ON RESPIRATORY ACTIVITY AND HISTOLOGICAL APPEARANCE OF ISOLATED PANCREATIC TISSUE

TREATMENT OF TISSUE	EXPERIMENT A		EXPERIMENT B	
	$\frac{Q_O_2 \text{ max.}}{Q_O_2 \text{ control}} \times 100$	Zymogen content of the cells	$\frac{Q_O_2 \text{ max.}}{Q_O_2 \text{ control}} \times 100$	Zymogen content of the cells
Control straight in fixative . . . . .		++		
Control in saline . . . . .	100	++	100	++
Pancreozymin 3.0 mg. % . . . . .			118	+
Pancreozymin 30.0 mg. % . . . . .			143	+
Pancreozymin 3.0 mg. % . . . . .	120	+		
Secretin 2.0 mg. % . . . . .	134	+		
Pancreozymin 30.0 mg. % . . . . .			136	+++ (scattering of granules)
Secretin 2.0 mg. % . . . . .	143	++		
Secretin 0.2 mg. % . . . . .				
Secretin 2.0 mg. % . . . . .	190	++		

TABLE 3. EFFECTS OF PANCREOZYMIN AND SECRETIN ON RESPIRATORY ACTIVITY OF ISOLATED PANCREATIC TISSUE

TREATMENT OF TISSUE	$\frac{Q_O_2 \text{ MAX.}}{Q_O_2 \text{ CONTROL}} \times 100$		
Pancreozymin 3.0 mg. %	153	118	153
Pancreozymin 10.0 mg. %	116		
Pancreozymin 30.0 mg. %	147 114	143	148
Pancreozymin 3.0 mg. %, Secretin 0.2 mg. %	120	136	136
Pancreozymin 30.0 mg. % Secretin 2.0 mg. %	134	142	144
Secretin 0.2 mg. %	143 109 112	136 111 106	120 109
Secretin 2.0 mg. %	190 111	110	133

ation in the histological appearance of the cells was observed. In the remaining 4 experiments where 1.0 mg. per cent of histamine was employed, there was no effect in one and a slight increase in respiration in the other 3 (104, 105, 130; control = 100). In one experiment alone, slight diminution in zymogen granules was observed.

*Gastrin.* Four experiments were carried out with concentrations of gastrin

of 0.6, 6.0 and 12.0 mg. per cent. Small increases in the rate of respiration were observed at the higher concentrations in 2 experiments (116 and 106; control = 100). At the highest concentration a slight decrease in zymogen granules was observed.

*Experiments in the absence of oxygen.* In 11 experiments the anaerobic glycolysis was from 3.0 to 4.5 (average 3.7, standard deviation 0.52). In such experiments the rate of glycolysis and the histological appearance of the cells was unaffected by the addition of acetyl choline with eserine, either alone or with ATP in (final) concentrations varying from 0.00001M to 0.01M.

#### DISCUSSION

The observations of Deutsch and Raper (1) that acetyl choline and secretin increase the rate of respiration of isolated cat pancreas have been confirmed. These experiments show that isolated pancreatic tissue can also be used to study the histological changes caused by a variety of agents. The results show that pancreozymin and pancreozymin-free secretin act as respiratory stimulants: the zymogen granules in the cells were decreased by the addition of pancreozymin or of acetyl choline with eserine but not by secretin. The slight stimulant effect of gastrin on the respiration of pancreatic tissue may be due to traces of pancreozymin in the preparation. Atropine does not prevent the increase in respiration following the addition of pancreozymin but completely inhibits both the respiratory and histological changes due to acetyl choline. These observations confirm those of Harper and Mackay (2).

Experiments showed that secretion does not occur in the absence of oxygen. The fact that the addition of ATP to the anaerobic tissue failed to cause secretion does not prove that ATP plays no part in the process since no evidence is available that this compound entered the pancreatic cells.

#### SUMMARY

A correlation has been made of the effects on respiration and on the histological appearance of cat pancreatic tissue stimulated *in vitro* by secretin, pancreozymin and acetyl choline with eserine. Secretion of zymogen granules was initiated and respiration stimulated by acetyl choline with eserine and by pancreozymin. Atropine inhibits the actions of acetyl choline but not those of pancreozymin. Secretin stimulates respiration but does not promote secretion of zymogen granules.

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# COMPARISON OF RESPIRATION AND GLYCOLYSIS IN THE BRAINS OF NORMAL AND FEBRILE RABBITS<sup>1</sup>

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THE maintenance of the body temperature of homoiotherms at a fairly constant level by operation of the hypothalamic temperature regulating centers implies that some temperature-sensitive process within the neurons of the centers must be so organized as to yield the equivalent of the set of a thermostat. The nature of this process is not known. The hypothesis that the rate of a key temperature-sensitive intracellular process is a factor determining this set level is under investigation in this laboratory.

The rise in body temperature following the administration of pyrogenic agents is commonly attributed to an elevation of this set level. According to the hypothesis stated above, the rate of the key metabolic process should be altered in the brain tissue of febrile animals. Since the majority of the energy-yielding processes now recognized influence measurements of oxygen consumption, glycolysis or both, these metabolic processes have been chosen in our search for a possible key reaction. Technical difficulties precluded direct investigation of the hypothalamic tissue. However, because of the qualitative similarity of the metabolic pattern in the several parts of the central nervous system (1), quantitative changes appearing in one region might well be paralleled by changes in a similar direction in other regions. Accordingly we have investigated the rates of respiration and of anaerobic glycolysis in cerebral cortex slices from normal rabbits and from rabbits with fever produced by the administration of a bacterial pyrogen. The results are presented in this paper.

## METHODS

Adult white rabbits weighing from 1.8 to 2.7 kg. were used. The animals were killed by injection of air into the marginal ear veins. The brain was rapidly removed and cerebral cortex slices were prepared by the cold moist box technique which has been described previously (2, 3). This procedure keeps the tissue in a cold moist environment from the time of excision until the respirometers are placed in the constant temperature bath. Thus in studies on oxygen consumption, imbalance between the aerobic and anaerobic phases of metabolism is minimized during the period of tissue manipulation (2, 4, 5), and in anaerobic work the overall metabolism is kept at a low level until suitable conditions are provided for the supply of nutrients and the removal of metabolic end-products (2). The wet weight of most tissue sam-

Received for publication February 14, 1949.

<sup>1</sup>This investigation was carried out under a contract between the Air Materiel Command, Wright-Patterson Air Force Base, Dayton, Ohio and Stanford University.

ples was 40 to 60 mg. Control experiments have shown that under the conditions of these experiments oxygen consumption is proportional to the initial wet weight of tissue over the range 10 to 90 mg. Slice thickness was 0.4 to 0.5 mm. (cf. 2). Immediately after weighing, tissue samples were placed in respirometer flasks or in small weighing bottles. The latter group of samples was dried to constant weight in an electric oven at 105°C.

Respiration was measured by the Warburg manometric method (2, 6, 7). The gas phase was oxygen. The liquid phase was Krebs' Ringer's phosphate (8) containing 0.011 M glucose. The center wells of the flasks contained 5 per cent KOH with Whatman no. 40 filter paper wicks. Manometric measurements were made in a constant temperature bath at  $39.0 \pm 0.01^\circ\text{C}$ . after 15 minutes of thermoequilibrium. Readings were taken at 10-minute intervals for a minimum of 60 minutes. Oxygen consumption was constant during this time. Results are expressed in the conventional 'Q' notation (cf. 2). Thus  $Q_o$  denotes microliters of oxygen consumed, measured under standard conditions, per mg. initial dry weight of tissue per hour.

Anaerobic glycolysis was measured by the manometric method (6, 7). The gas phase was 95 per cent  $\text{N}_2$ -5 per cent  $\text{CO}_2$  which had been passed through a Savage-Ordal (9) hot reduced copper tube to remove traces of oxygen. Uniform gassing was effected by passing this mixture through the respirometers in series. After leaving the last respirometer the gas was passed through a Wolff bottle so that the rate of flow could be observed and maintained. Gassing was carried out for a minimum of 10 minutes after the respirometers had been placed in the constant temperature bath. The liquid phase was Krebs-Henseleit (10) bicarbonate solution. This medium had previously been gassed for over an hour with the same oxygen-free mixture used as the gas phase in the respirometers. Manometric measurements were made in a constant temperature bath at  $39.0 \pm 0.01^\circ\text{C}$ . Readings were taken at 10-minute intervals for 40 to 60 minutes. The rate of glycolysis was constant during this time. Results are expressed in the conventional 'Q' notation. Thus  $Q_A^N$  denotes microliters of acid produced, measured as a gas under standard conditions, per mg. initial dry weight of tissue per hour. All Q values given in tables were calculated from measurements made during the period of steady state.

Fever was induced in those animals referred to as 'febrile' by injection of 0.05 ml. of sterile typhoid-paratyphoid triple vaccine<sup>2</sup> (hereinafter termed T.P.T.) into the marginal ear vein. Rectal temperature was followed (taking care not to disturb the animals, cf. 11) for an hour. No animal was used unless rectal temperature rose at least 1°C. in this time. All febrile animals were killed exactly one hour after injection of T.P.T.

#### RESULTS

Table 1 shows the results of measurements of oxygen consumption in normal and in febrile rabbit cerebral cortex slices at 39°C. The mean values of  $Q_o$  are 8.76 and 8.58 respectively. The significance of the observed difference between these

<sup>2</sup>This vaccine, prepared by the Cutter Laboratories, Berkeley, California, contained in each ml. 1000 million *E. typhosa*, 500 million *S. paratyphi* and 500 million *S. schottmuelleri*. The preservative was 0.25 per cent tricresol.

two means was evaluated by the use of Student's 't'-test (cf. Snedecor, 12). The values of  $t$  and  $P$  obtained were 0.659 and approximately 0.5 respectively. Thus there appears to be no significant difference in respect of rate of oxygen consumption at 39°C. between cerebral cortex slices from normal and from febrile rabbits.

The results of a similar comparison between the rates of anaerobic glycolysis in cerebral cortex slices from normal and febrile rabbits at 39°C. are shown in table 2. Here again it was found by application of Student's 't'-test that the observed difference between the means was not significant.

Comparison of the variances (squared standard deviations) by the "F" or variance ratio method (cf. 12) indicates that there is no significant difference in the variability of  $Q_O_2$  and of  $Q_A^{N_2}$  between the normal and febrile rabbits.

TABLE 1. COMPARISON OF OXYGEN CONSUMPTION AT 39°C. OF CEREBRAL CORTEX SLICES FROM NORMAL AND FEBRILE RABBITS (FOUR ANIMALS (12 TISSUE SAMPLES) IN EACH GROUP)

	NORMAL	FEBRILE
Mean $Q_O_2$ . . . . .	8.76	8.58
Range: $Q_O_2$ . . . . .	7.83-10.15	7.67-9.63
Standard deviation . . . . .	0.690	0.646
No. of observations . . . . .	12	12

Comparison of means:  $t = 0.659$ ;  $P = 0.5$ .

TABLE 2. COMPARISON OF ANAEROBIC GLYCOLYSIS AT 39°C. OF CEREBRAL CORTEX SLICES FROM NORMAL AND FEBRILE RABBITS (SIX ANIMALS (48 TISSUE SAMPLES) IN EACH GROUP)

	NORMAL	FEBRILE
Mean $Q_A^{N_2}$ . . . . .	12.58	12.27
Range: $Q_A^{N_2}$ . . . . .	8.49-21.13	7.88-19.53
Standard deviation . . . . .	2.590	2.561
No. of observations . . . . .	48	48

Comparison of means:  $t = 0.590$ ;  $P = 0.5$ .

#### DISCUSSION

These results show that the altered thermostatic behavior by which a bacterial pyrogen appears to induce fever in the rabbit is not accompanied by alteration of either the oxygen consumption or anaerobic glycolysis rates, at 39°C., of cerebral cortex tissue removed one hour after the injection of the pyrogen. Thus these findings lend no support to the concept presented in the introduction to this paper that the cellular metabolism of the central nervous system is a factor determining the level at which the thermoregulatory processes operate. However they do not necessarily invalidate this hypothesis for the following reasons: *a*) The pyrogen may influence thermostatic behavior by affecting processes other than those determining the rates of oxygen consumption or anaerobic glycolysis. *b*) The assumption of qualitative similarity of metabolic pattern in the several parts of the central nervous system may be incorrect, the pyrogen affecting hypothalamic but not cortical tissue metabolism. *c*) The pyrogen might produce metabolic effects *in vivo* which are lost when tissue slices are studied *in vitro*. *d*) A possible metabolic effect of the pyrogen may have appeared and waned by the end of the first hour. Grant (13) has shown that the vasoconstriction and suppression of thermal polypnea (which

are the principal causes for the febrile temperature rise in the rabbit) are replaced by vasodilatation and polypnea at approximately one hour after injection of T.P.T., the time at which the animals were killed for the metabolic studies reported here. However this objection is weakened by the finding that some 30 minutes later vasoconstriction and polypnea suppression return without further pyrogen administration. Further, during the period of vasodilatation and polypnea, the respiratory rate may not be as high as it was before injection in spite of the presence of a two degree rise in rectal temperature, which suggests that the factor (presumably the pyrogen) depressing the respiration is still active.

In view of these considerations the possibility that thermoregulatory behavior may be related to the metabolic activity of the central nervous system remains an open question.

#### SUMMARY

The mean  $Q_o$  values at  $39^{\circ}\text{C}$ . for cerebral cortex slices from normal rabbits and from rabbits with fever produced by administration of typhoid-typhoidpara vaccine were 8.76 and 8.58 respectively. The difference between these means is not statistically significant. The mean  $Q_A^N$  values at  $39^{\circ}\text{C}$ . for cerebral cortex slices from normal and febrile rabbits were 12.58 and 12.27 respectively. The difference between these means is not statistically significant.

An hypothesis relating the level at which body temperature is regulated to the metabolic activity of the central nervous system is stated and the bearing of these findings on that hypothesis is discussed.

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# INFLUENCE OF ANTIPYRINE ON RESPIRATION, GLYCOLYSIS AND CHOLINESTERASE ACTIVITY IN RAT BRAIN<sup>1</sup>

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**I**N THE introduction to the preceding paper in this volume an hypothesis relating thermostatic behavior of homoiotherms to central nervous system function was set forth. In this paper are reported experiments designed to subject this hypothesis to a further test by determining whether antipyrine, a substance reducing body temperature, affects certain metabolic activities of central nervous system tissue.

## METHODS

Adult albino rats of the Slonaker-Wistar strain were used. The procedures employed in the preparation of cerebral cortex slices and for the measurement of respiration and glycolysis were the same as described previously (1) except that the animals were killed by decapitation rather than by intravenous injection of air as in the case of the rabbits. When oxygen consumption was measured the sidearms of the respirometer flasks contained graded amounts of antipyrine (Merck) made up in the same Krebs-Ringer's phosphate glucose solution (2) used as the suspension medium. The contents of the flasks were added to the main compartments of the flasks after a 30-minute control period (which followed 15 minutes of thermoequilibration), so that control and 'antipyrine added' runs were made with the contents of each flask. This procedure was not followed in the measurement of glycolysis because the handling necessary to make additions from the vessel sidearms disturbed the otherwise even rate of carbon dioxide evolution. In order to avoid this effect the desired concentrations of antipyrine were made up in the Krebs-Henseleit bicarbonate glucose medium (3) used in the main compartments of the flasks. Thus there was no 'pre-addition' control period as in the case of the respiration measurements and comparisons were made against controls containing no antipyrine in the liquid phase.

Cholinesterase activity was measured manometrically (4-6). The general procedure has been described previously (6). The enzyme extract used was the 'super-natant fraction' of Nachmansohn and Feld (7). This was prepared from whole brain homogenized in cold ( $0^{\circ}\text{C}$ .C.) calcium-free Krebs-Henseleit bicarbonate solution, together with the desired amounts of antipyrine. The sidearms contained acetylcholine chloride (Merck) made up in the same solution. The contents of the side-

Received for publication February 14, 1949.

<sup>1</sup> This investigation was carried out under a contract between the Air Materiel Command, Wright-Patterson Air Force Base, Dayton, Ohio and Stanford University.

arms were added to the main compartments of the flasks at the end of thermoequilibration. The final concentration of acetylcholine was  $0.015\text{ M}$ . The gassing procedure was the same as in the experiments on anaerobic glycolysis (1). The  $\text{pH}$  of the liquid phase in the flasks, in equilibrium with the gas mixture 95 per cent  $\text{N}_2$ -5 per cent  $\text{CO}_2$ , was 7.4.

All of the manometric measurements were made in a constant temperature bath at  $37.5^\circ \pm 0.01^\circ\text{C}$ . When oxygen was the gas phase the respirometers were gassed before being placed in the water bath. When the 95 per cent  $\text{N}_2$ -5 per cent  $\text{CO}_2$  mixture was used gassing was accomplished during the 15 minute thermoequilibration period which preceded all runs.

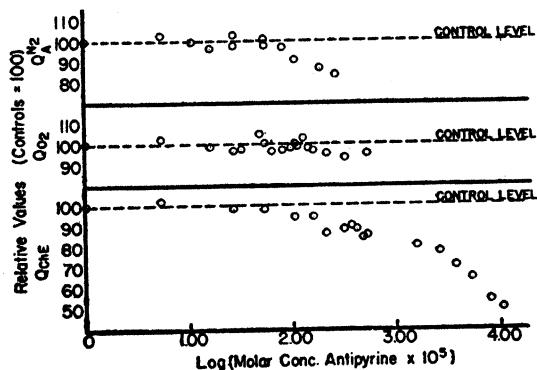


Fig. 1. GRAPH SHOWING EFFECT OF GRADED CONCENTRATIONS of antipyrene on oxygen consumption and anaerobic glycolysis in rat cerebral cortex slices and on cholinesterase activity in the supernatant fraction of whole rat brain homogenate at  $37.5^\circ\text{C}$ .

Respiration and glycolysis data are expressed in the conventional 'Q' notation on the initial dry weight basis (cf. 1). In all cases the oxygen consumption was constant for several hours and glycolysis for 40 to 60 minutes. Values given in the figure and table were calculated from readings made during steady state periods. Cholinesterase activity is expressed in terms of milligrams of acetylcholine hydrolyzed in one hour by 1.0 ml. of supernatant fraction from an homogenate containing 100 mg. of fresh whole rat brain per ml. The symbol  $\text{QchE}$  is used to denote this (cf. 8). Readings were taken at 5-minute intervals for a period of 40 minutes. The rate of  $\text{CO}_2$  evolution was constant during this time.

## RESULTS

*Anaerobic glycolysis.* Figure 1 shows the effect of antipyrene on the rate of anaerobic glycolysis in rat cerebral cortex slices. Concentrations ranging up to about  $5.3 \times 10^{-4}\text{M}$  had no effect. With further rise in antipyrene concentration an inhibition was observed which amounted to 17 per cent at the highest concentration used ( $2.6 \times 10^{-3}\text{M}$ ). There is no indication of an augmentation phase of anaerobic glycolysis caused by antipyrene such as has been observed when the effect of graded concentrations of another antipyretic, magnesium chloride, was investigated (10).

*Respiration.* It is shown in figure 1 that the respiration of rat cerebral cortex slices was not affected by antipyrene up to a concentration of about  $2 \times 10^{-3}\text{M}$ . With further increase in antipyrene concentration a slight inhibition of oxygen con-

sumption was observed. However this amounted to only 5 to 7 per cent of the highest concentration of the drug tested ( $5.3 \times 10^{-3}M$ ). Thus the respiratory process in rat cerebral cortex resembles that of rat liver suspensions (9) in respect of stability toward antipyrine.

*Cholinesterase activity.* It is shown in figure 1 that antipyrine, in concentrations up to about  $5.3 \times 10^{-4}M$  has no effect on the cholinesterase activity of the supernatant fraction of rat brain homogenate. With increasing concentrations of the drug inhibition develops. Fifty per cent inhibition was observed at the highest concentration studied,  $10.6 \times 10^{-2}M$ . Thus our rat brain cholinesterase preparation was considerably less sensitive toward antipyrine than human serum cholinesterase, with which 50 per cent inhibition occurred at a concentration of antipyrine less than one tenth as great (11, 12).

*Control experiments: Statistics.* A considerable number of control experiments were run in the course of this investigation. The data so obtained provide values of  $Q_O_2$  and  $Q_{A^N_A}$  of rat cerebral cortex slices and of the cholinesterase activity of the

TABLE I. MEANS AND OTHER STATISTICAL DATA OF CONTROL EXPERIMENTS ON RESPIRATION AND ANAEROBIC GLYCOLYSIS IN RAT CEREBRAL CORTEX SLICES AND CHOLINESTERASE ACTIVITY IN THE SUPERNATANT FRACTION OF WHOLE RAT BRAIN HOMOGENATE; 37.5°C.

	$Q_O_2$	$Q_{A^N_A}$	ACb HYDROLYZED/HOUR/1.0 ML. SUPERNATANT
Mean.....	11.13	9.26	2.63
Range.....	9.85-12.71	5.04-12.76	2.40-2.89
Standard deviation.....	0.229	1.685	0.126
No. of runs.....	30	27	15

For description of units see text.

supernatant fraction of whole rat brain homogenate at 37.5°C. which may be of some use to other workers in the field of tissue metabolism. The absolute values of the mean rates of these processes together with certain derived statistics are given in table I.

#### DISCUSSION

The bearing of these results on the hypothesis concerning the mechanism of temperature regulation formulated in the introduction to this paper may now be considered. The intravenous dose of antipyrine which in the rabbit causes increased respiration, dilatation of ear vessels and a fall in rectal temperature ranges from 0.06 to 0.25 gm./kg. body weight. Data concerning the resultant blood concentrations are not available, but on the assumption that antipyrine is uniformly distributed throughout the body these doses would yield concentrations from  $3.2 \times 10^{-4}M$  to  $1.3 \times 10^{-3}M$ . Such concentrations, according to the findings reported in this paper, are somewhat below the minimum required for depression of oxygen consumption *in vitro*. However, the concentrations estimated to result from the highest doses are within the range in which some depression of anaerobic glycolysis and cholinesterase activity of brain tissue might occur.

It may be noted that magnesium chloride, which also causes increased respiration, dilatation of ear vessels and lowered body temperature (13), has an effect on the rate of anaerobic glycolysis in cerebral cortex tissue in the direction opposite to that of antipyrine (10). Magnesium chloride, in the concentration range concerned, resembles antipyrine in leaving the rate of oxygen consumption unaffected.

As far as conclusion from a study of the two agents, antipyrine and magnesium, may be warranted, it would appear that activation of heat defense mechanisms by a drug bears no constant relation to its influence on anaerobic glycolysis in nervous tissue and can occur in the absence of any change in oxygen consumption.

While it is obvious that these considerations lend no support to the hypothesis under discussion (see introduction), the limitations on the applicability of this type of metabolic data to the evaluation of that hypothesis, which have been set forth in the preceding paper, are such that we consider the hypothesis to warrant further testing.

#### SUMMARY

Antipyrine, when added to the medium in which slices of rat cerebral cortex are suspended, has no effect on the rate of oxygen consumption at 37.5°C. in concentrations below  $2 \times 10^{-3}$ M. At concentrations greater than this, slight depression of respiration occurs. Anaerobic glycolysis in brain slices and cholinesterase activity in brain homogenates are depressed by concentrations of antipyrine above  $1 \times 10^{-3}$ M. The question of the possible bearing of these metabolic effects of antipyrine on its antipyretic action is discussed.

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# ACTION OF METHYL FLUOROACETATE ON RESPIRATION AND POTENTIAL OF NERVE<sup>1</sup>

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**T**HREE is general agreement that pyruvate is a key link in carbohydrate oxidation and glycolysis. Whether the further oxidation of pyruvate is via a simple decarboxylation or through the Krebs cycle is not known for nerve. Although glucose is burned and fermented by resting nerve, the excess metabolism of activity may involve phospholipid breakdown (1). Energy from these reactions serves to build energy-rich phosphate bonds, stored in nerve mostly as CrP (2), which probably are the more immediate energy source for maintaining cell integrity and function. The fluoroacetates are known to interfere with pyruvate oxidation, and perhaps with that of acetate separately (3), to interfere in the Krebs cycle, and to block specifically the extra oxygen consumption of activity in the case of muscle (4); as does azide in muscle (5) and nerve (6, 7). Correlated studies on the influence of this inhibitor on the respiration and functional properties of nerve should thus yield information as to the metabolic basis of nerve activity.

## METHODS

Sciatic nerves of summer, winter and spring green frogs, with perineurium slit to facilitate diffusion, were mounted singly in a three-compartmented chamber. Electrodes of Ag-AgCl or of Pt were arranged as follows: in an end compartment, two stimulating electrodes; in the middle one, two pick-up electrodes (leads A and B); in the far end one, a pick-up (lead C) and an indifferent electrode. Dimensions are shown in figure 5. The central compartment was kept filled with Ringer's or a drug solution, substitution being made without moving the preparation. Stimulation, normally only during measurements, was at 20 per second by a conventional thyratron stimulator with a transformer output. Action potentials were measured on the cathode ray tube face for height or photographed or traced for study of shape. Experiments were begun within half an hour of killing the frog and spike heights normally remained constant for over 6 hours. A 30- to 60-minute control period always preceded the application of test solutions. Electrical studies were made at room temperature, 21° to 28°C. in various experiments.

Respiration studies were carried out in the microrespirometer previously described (8) or, in stimulation experiments, in a new modification (7). For resting  $Q_{O_2}$ , 3-mg. stretches of nerve were run at 30°C. (summer, 1947); for active  $Q_{O_2}$ , 10-mg. stretches (summer, 1948). Solutions were made in Ringer-bicarbonate, reagent being substituted for a molar equivalent of NaCl when any serious departure from isotonicity was involved, and adjusted to  $pH$  6.8-7.4. The MFA was tested for purity by boiling point determination.<sup>3</sup>

Received for publication January 31, 1949.

<sup>1</sup> Performed under contract between the Office of Naval Research and the University of Chicago. A preliminary report appeared in *Federation Proc.* 7: 11, 1948.

<sup>2</sup> Predoctoral Public Health Fellow.

<sup>3</sup> We are indebted to Dr. J. O. Hutchens, Toxicity Laboratory of the University of Chicago, for the fluoroacetates.

## RESULTS

*Sodium fluoroacetate* is almost inert for frog nerve. Spike height was unaffected at concentrations up to 100 mM, with the nerve left mainly at rest or, compared to a control, when tetanized continuously for 13 hours. Even at pH 6.0, which should

TABLE I

INHIBITOR	% INHIBITION OF RESPIRATION		
	2 hrs.	3 hrs.	4 hrs.
<i>Sodium fluoroacetate</i>			
0.01M .....	-8 (6) <sup>1</sup>	3	7
0.10M .....	(3)	40	55
<i>Methyl fluoroacetate</i>			
0.005M .....	80 (6)	80	80
0.001M .....	25 (3)	30	35

<sup>1</sup> Number of experiments indicated in ( ).

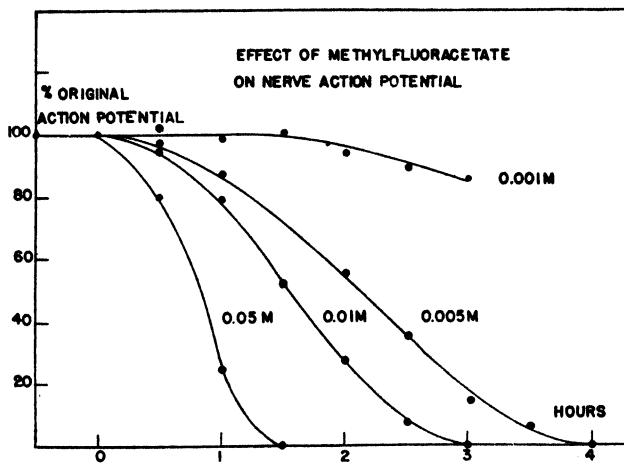


Fig. 1

favor penetration by un-ionized molecules, no action potential changes were produced.

Respiration of paired nerves agreed within 10 per cent ( $Q_{O_2}$  at  $30^{\circ}\text{C}.$  =  $142 \pm 29$  in 36 experiments), and no change resulted from soaking as long as 4 hours in 10 mM Na fluoroacetate (table I). At 100 mM, oxygen consumption was reduced 50 per cent, the spike remaining normal. The feeble action of this substance is, therefore, not entirely due to lack of penetration; a conclusion supported by its similar lack of activity in inhibiting dehydrogenases in nerve or brain homogenate (9).

*Methyl fluoroacetate* (*MFA*), in contrast, blocks nerve in concentrations lower than does DFP (10, 11), though not so low as cyanide (12) or IAA (13). One mM

solutions are ineffective, but at 5 mM block is complete in 3 to 4 hours and at 50 mM, within 1.5 hours (fig. 1). Continued tetanization does not alter the curve of fall, in contrast to the IAA-poisoned nerve (13). Spike height falls along a sigmoid curve, as if a latent period exists in the action of MFA (but see thresholds, below), longer with lower concentration; but the shape of the curve is more directly determined by the order of failure of individuals in the fiber population. Thus, spike height at



Fig. 2

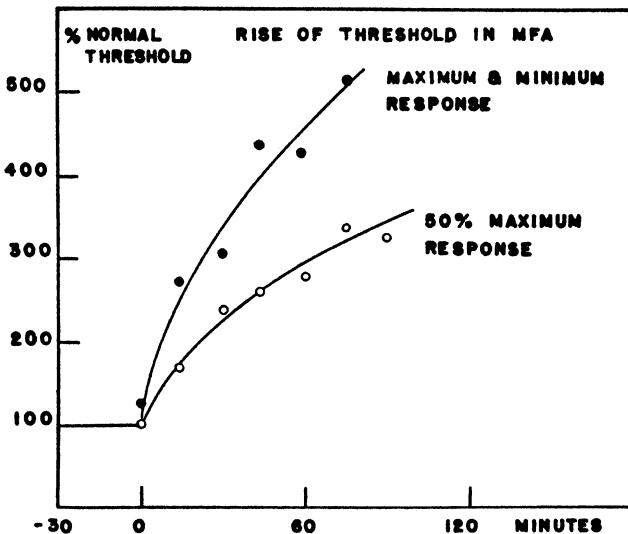


Fig. 3

the C electrode, beyond the exposed stretch of nerve, falls with or slightly ahead of that at the A electrode on the exposed region. Further, at the B electrode, more distal in the middle compartment, the potential falls with that at C, while at A, more proximal, the fall is less rapid. A particular fiber, then, may block anywhere along its exposed length but its spike remains essentially at full value up to the point and time of block. Changes in shape of the nerve action potential (fig. 2) show a decrease of conduction velocity paralleling a rise in threshold before fibers begin to block. Block then develops progressively from the larger to the smaller fibers, without further slowing. Threshold, for minimal, 50 per cent, or maximal spike response, begins to rise at once and increases 2- to 5-fold over 30 to 90 minutes, alike in either 0.005 or 0.01 M MFA (figure 3). No change (or a slight increase in some

cases) in demarcation potential, measured at the nerve end in MFA (.01 or .005 M) compared to an end in saturated KCl, was observed up to complete block.

Winter frog nerves are only about half as susceptible to MFA as are summer nerves. Five mm blocks in 5.5 to 6 hours in winter, as compared to 3 to 4 hours in summer, and the 'latent period' is about 2 hours. At 10 mm concentration, the fall begins, in winter, in 1 to 1.5 hours and block is complete in 2.5 to 3 hours.

Once spike height has begun to fall, washing in Ringer does not alter its course. The inhibition is, therefore, irreversible after exposure for an hour or so. This is different from the inhibition of CrP resynthesis in muscle, which is reversible on washing (4). In yeast a partial reversal of the inhibited respiration is obtained with acetate (14), and in chilomonas with pyruvate and alcohol (15). We have tested a number of substrates for their ability to prevent or reverse MFA inhibition of nerve.

The oxygen consumption of nerve is more sensitive to MFA than is conduction. Even 1 mm cuts respiration by 30 per cent, with no block, and 5 mm reduces respiration to 20 per cent of normal at a time when only half the fibers are blocked (table 2). This re-emphasizes the factor of safety (16) in the energy supply of nerve, seen also

TABLE 2

HRS. AFTER MFA (0.005M)	% OF NORMAL FOR FROG SCIATIC			
	No addition		Na Fumarate (0.05M)	
	Action potential	Respiration	Action potential	Respiration
1	85 (5)	(6)	100 (5)	(3)
2	50	20	100	45
3	15	20	100	55
4	0	20	100	

under conditions of hypoxia. The increased oxygen consumption on tetanization may be as much in nerves poisoned with MFA (1 to 5 mm) as in normal controls at times when the resting respiration of the MFA nerves has been cut as low as half normal. (Details will be published elsewhere, 7.) Addition of Na fumarate to normal nerve, up to 100 mm, has no effect on oxygen consumption; but added an hour before MFA, at ten-fold the molarity of the inhibitor, it reduces the inhibition from 80 per cent to only 50 per cent (table 2, fig. 4). With this increment in metabolism comes complete prevention of block.

In action potential experiments, each substrate was added an hour before the MFA (in similar substrate solution) and was tested in concentrations of 2 and 5 times that of the inhibitor (5 mm in summer, 10 mm in winter). Glucose, ethyl alcohol, acetate, pyruvate and alpha ketoglutarate were completely ineffective against MFA. Malate and perhaps oxaloacetate gave partial protection in 5:1 concentration, succinate protected fully at 5:1, partially at 2:1 (fig. 5). Most striking is the action of fumarate, which gave full protection for over 6 hours at twice the molarity of MFA (fig. 5.) This protection by fumarate can still be attained when it is added within 15 minutes after MFA, while after 30 minutes it is completely ineffective. This is further striking evidence of a brief 'latent period' in MFA action during which

its effects can be reversed, and of a succeeding period when damage has become irreversible even though conduction has hardly begun to fail. (That MFA action is not delayed by slow penetration is shown by the prompt increase in threshold.)

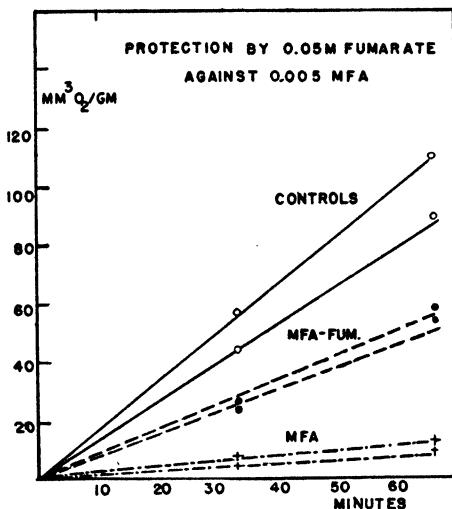


Fig. 4

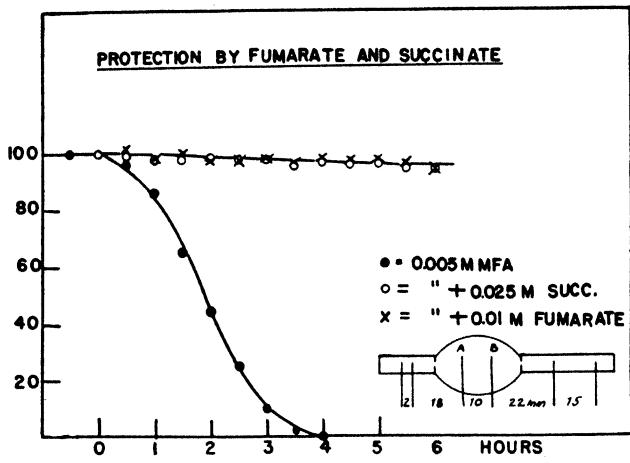


Fig. 5

This also speaks against MFA acting as a competitive inhibitor; although, on the other side, 20 mm fumarate protected against 10 mm MFA and 40 mm against 20 mm, but 20 mm fumarate did not protect against 20 mm MFA.

Controls with substrates on normal nerve showed them inactive—spike height

remained normal in 10 mM Na acetate for over 6 hours—except for malate and glucose added to Ringer in high concentration. The former, 100 mM, blocked conduction in two hours and even at 50 mM caused spike height to fall to half within 30 minutes, where it remained. Glucose, 100 mM, led to an increase in spike height of 15 to 20 per cent, which was maintained for hours. An increase followed by block, reported by others (17, 18) was probably due to fuller loss of electrolyte.

#### DISCUSSION

A nerve blocks, presumably, when the eddy currents between active region and to-be-activated region do not reach the threshold of the latter. This results, for unchanged geometry and conducting media, when the action spike is sufficiently decreased or the membrane threshold is sufficiently raised. While, in general, membrane potential decreases along with spike height, the two can vary independently. Similarly, membrane threshold and potential do not necessarily vary in parallel fashion. MFA blocks conduction by raising threshold (and slowing conduction), without altering the demarcation potential or the action spike. This resembles the narcotics (19, 20) or DFP (11), which also raise the nerve threshold; but not cyanide (19-21), anoxia (22), or IAA (23), which depolarize. With MFA, the threshold rise starts at once and is twice normal in 15 minutes, when conduction is slowed but otherwise unaltered. Even at 30 minutes, when the threshold is increased threefold, the action potential is over 95 per cent normal. A factor of safety of at least three is thus shown.

MFA does not decrease the metabolism of activity independently of that of rest. On the contrary, the extra oxygen consumption of conduction may be normal when the resting oxygen consumption has been cut below one-third, though it also decreases at higher MFA concentration before conduction fails. This is in sharp contrast to the action of azide, which can abolish the extra oxygen consumption of activity with disturbing resting respiration or conduction (6, 7), as well as to the action of MFA on muscle. Resting oxygen consumption, on the other hand, is depressed by MFA in concentrations and at times which still leave conduction intact. Even fumarate, which protects conduction fully against MFA block, leaves the resting oxygen consumption 50 per cent depressed by MFA. Clearly a factor of safety in the metabolic flow of energy exists, as earlier indicated (16). This result also suggests that MFA block of metabolism occurs above some energy-yielding steps, which continue to supply energy for function when oxygen uptake, nearer the top of the sequence, has been cut.

Metabolic energy, perhaps both resting and active together, is funneled into functional use, in nerve as in other tissues, through CrP and ATP (2). Interference with oxidations or glycolysis or both decreases the CrP content, but oxidative energy is the more important in maintaining normal conditions. Fluoracetate acts as an inhibitor in the oxidative chain, just where is not fully agreed upon, but does not decrease lactic acid formation (4), and it especially prevents CrP resynthesis and recovery oxygen consumption (4) and heat production (24) in muscle. (Metadinitrobenzene may decouple oxidations and phosphorylations, 23; and methylene blue, which, like MDB, markedly diminishes the creatin phosphate of muscle,

2, perhaps acts similarly. This action is not excluded for MFA.) Since the resting, but not the active, metabolism of nerve depends largely on carbohydrate oxidation (25), while the increase on activity is perhaps associated with phospholipid loss, it was thought that MFA, supposed at first to block acetate oxidation (3), might differentially depress the active metabolism in nerve even more than in muscle. This it does not do; and fuller evidence on MFA action (26, 3, 14) implicates pyruvate oxidation, presumably via the Krebs cycle (4). The sharp differences in the behavior of nerve and muscle—NaFA inactive on nerve (Dr. Stannard has, however, noted a similar inactivity on muscle), MFA action irreversible, and resting rather than active metabolism inhibited—emphasize again fundamental metabolic differences between these tissues.

That the Krebs cycle is operative in brain, is now well supported (27-29). In the case of nerve, the depressant action of arsenite (30), known to inhibit the oxidative decarboxylation of alpha ketoglutaric acid (31), suggests the cycle; while the failure of malonate, which inhibits the dehydrogenation of succinic acid, to depress resting potential (23) or spike, as we have found, speaks against it. The negative results cannot be dismissed simply as lack of penetration, since malonate is also relatively ineffective on the dehydrogenases of nerve brei (9). None the less, our findings that the tricarboxylic intermediates, especially fumarate, can prevent MFA block and counter its inhibition of respiration, speak for the presence and functional importance of a tricarboxylic cycle in nerve.

#### SUMMARY

Na fluoroacetate is almost inactive on frog sciatic nerve. Conduction is unaffected at 100 mm concentration and oxygen consumption is only 50% depressed. Lack of penetration does not adequately account for this feeble activity. Methyl-fluoroacetate interferes with resting metabolism and leads to irreversible conduction block, large fibers before small ones, after a 'latent' period during which threshold is rising. In summer frogs, more sensitive than winter ones, 5 mm MFA blocks conduction in 3 to 4 hours by raising the threshold 2- to over 5-fold and slowing conduction 2-fold or more. Resting potential and spike height, for individual fibers, are not altered. Resting oxygen consumption is cut to 70 per cent normal by 1 mm MFA, to 20 per cent by 5 mm; but the extra  $Q_{O_2}$  of activity may remain intact when the resting value is severely cut. Fumarate and succinate can protect against MFA action; ethanol, acetate, pyruvate, alpha-ketoglutarate, and glucose are ineffective.

These findings emphasize the safety factor in nerve metabolism and the metabolic difference between nerve and muscle, and support the existence and functional importance of a tricarboxylic cycle in nerve metabolism. The selective inhibition of resting rather than of active oxygen consumption, by MFA on nerve, is a unique reversal of the more usual selective inhibition of activity metabolism.

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# ACTION OF ANTICHOLINESTERASES, DRUGS AND INTERMEDIATES ON RESPIRATION AND ELECTRICAL ACTIVITY OF THE ISOLATED FROG BRAIN<sup>1</sup>

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**W**AR research has made available a number of new metabolic inhibitors with striking pharmacologic actions. Some of these are potent cholinesterase (ChE) inhibitors, as diisopropyl fluorophosphate (DFP) and the polyphosphonate esters, tetraethylpyrophosphate (TEP) or hexaethyltetraphosphosphate (HTP), and their action has been widely interpreted in terms of an accumulation of acetylcholine (ACh). Others do not affect ChE, as sodium or methyl fluoracetate (MFA), and their action has been ascribed to interference with oxidative sequences and energy yield. The functional importance of a particular chemical step can be explored by observing the effect on function of blocking that reaction, as in the classical work with IAA on lactic acid formation in muscle. We have, accordingly, studied the action of several of these inhibitors, alone and in combination with eserine, atropine, caffeine or Krebs' cycle substrates, on the isolated frog brain. This preparation yields a regular electric rhythm spontaneously and large recurrent traveling potential waves under the influence of strong caffeine, and these have been taken as an index of the physiological state of the tissue. As a further check on the overall metabolism, the oxygen consumption of bits of brain has been followed. A few assays of ChE inhibition of brain homogenate or slices have also been performed (with D. Luck). The physiological action of both types of inhibitors (MFA, DFP, TEP) has been found to follow more closely their inhibition of oxygen consumption than their inhibition of ChE.

## METHODS

Preparation of and recording from the isolated frog brain have followed the earlier description (1). Reagents were applied by soaking in the desired solution for a short time, usually 2 minutes, then dipping quickly into Ringer. When caffeine (0.5%) waves were being studied, caffeine (0.1%) was re-added with the test solution to prevent washing out of the alkaloid.

For respiration studies, the capillary respirometer (2) and the Warburg methods have been used. Bits of the primordium pallei, some 2 mm. long oriented from midline to lateral convexity, some 1 mm. wide from anterior to posterior, and 0.2 mm. thick from pial to ventricular surfaces, were used in the capillary. Each bit weighed about 0.7 mg. and experimental and control pieces were taken as far as practicable bilaterally from like regions of the hemispheres. In the Warburg experiments, 3 hemispheres, sliced into pieces roughly of the above size, were used in each manometer. Since, after completing the preparation, some time must elapse before satisfactory readings are

Received for publication January 31, 1949.

<sup>1</sup> Performed under contract between the Office of Naval Research and the University of Chicago. A preliminary report appeared in *Federation Proc.* 7: 15, 1948.

obtained,  $Q_{O_2}$  values are mostly given for the second hour after drug application. The brain bit was left in the desired solution for 2 to 6 minutes (less often 15 to 20, with like results), quickly washed, blotted, and mounted in the respirometer. Readings were normally begun half an hour later. Vapor pressure of the drugs themselves did not alter readings. Electrical measurements were made at room temperature, mostly 20° to 23°C.; respiration measurements in a bath at 25° to 28°C.

ChE assays were made by a standard frog rectus method after incubating ACh with brain homogenate. Manometric measurements supplemented these, 50 per cent inhibition by the various drugs being attained at some ten-fold lower concentrations. Figures are given for the muscle assay.

The drugs were obtained from the University of Chicago Toxicity Laboratory, (Dr. Hutchens), the Victor Chemical Works and Edgewood Arsenal (Dr. Hinwich), and were tested for purity by measurement of boiling point. Stock material was kept in a desiccator, solutions being made in Ringer bicarbonate (gm/l. NaCl 6.5, KCl .2, CaCl<sub>2</sub> .25, NaHCO<sub>3</sub> .15), adjusted to a pH of 7.0 to 7.5 before using. Results obtained, especially with TEP, were different quantitatively from one season to another but probably this variance is due to the frogs and not to the use of different batches of chemicals. Other differences, in critical drug concentrations and even type of electrical effect, were encountered when some of these experiments were repeated in the summer at another laboratory. The results reported are those regularly obtained here.

## RESULTS

*NaFA.* This agent was inactive, even in 50 mM concentration, on the normal or caffeine waves of frog brain or on its respiration. This inertness, despite powerful pharmacological action *in vivo* on mammals (2a, 3a), is not due entirely to lack of penetration, for NaFA proved similarly ineffective in slowing MB reduction by rat brain homogenate (3). Similar lack of activity for muscle has been observed (4). On intact peripheral nerve, also, NaFA did not depress conduction or respiration (5), although in other species marked depression of brain respiration has been found (6).

*MFA.* In contrast to the sodium salt, the methyl ester inhibits respiration by 10 per cent at 5 mM, by 45 per cent at 12 mM and by 80 per cent at 25 mM. At 0.1 to 2 mM, there may be some stimulation of O<sub>2</sub> consumption (table 1A). MFA also inhibits a number of dehydrogenases, as tested by MB reduction (3), and depresses conduction and respiration of nerve (5). ChE is 50 per cent inhibited at 10 mM. Action on brain potentials begins at 12 mM, which cuts amplitude to half without affecting frequency, the change being complete within 20 minutes (fig. 1). Higher concentrations produce the same changes, but more rapidly, until 100 mM, which abolishes all activity after a 2-minute soaking. After a one-minute exposure to this concentration, a very feeble rhythm of normal frequency remains.

In the presence of .5 per cent caffeine, 12 mM MFA inhibits respiration by only 20 per cent. Caffeine spikes are transiently increased in amplitude and frequency by this MFA concentration, which is threshold, but return to normal in a minute. Stronger solutions (16-50 mM) abolish all activity or cut both amplitude and frequency (to 50% by 25 mM exposed for half a minute, to zero in 4 minutes, exposed for 2 minutes). All the MFA effects on electrical waves can be reversed by prompt washing, but after a few minutes they are permanent.

*DFP.* At 1 mM concentration, DFP inhibits oxygen uptake by 10 per cent; at 10 mM, by 50 per cent (table 1B). ChE is inhibited about 50 per cent by 0.3

TABLE I. INHIBITION OF BRAIN OXYGEN CONSUMPTION

NUMBER OF EXPERIMENTS AVERAGED	DRUG CONCENTRATION (mM)	Q <sub>O<sub>2</sub></sub> (2ND HR.)		INHIBITION (%)
		Control	Drug	
<i>A. MFA</i>				
1	0.01	440	435	0
1	0.1	370	385	-4
1	2.	755	790	-5
2	5.	880	830	6
3	10.	565	310	45 (40-50)
2	12.	520	300	42 (32-52)
3	16.	365	140	62 (50-74)
1	25.	430	80	81
<i>B. DFP</i>				
1	0.01 <sup>1</sup>	385	390	0
1	0.5	580	330	43
1	1. <sup>1</sup>	305	265	13
1	3. <sup>1</sup>	180	160	12
2	5. <sup>1</sup>	345	235	32 (24-40)
1	6. <sup>1</sup>	240	140	45
1	10.	495	305	38
2	10. <sup>1</sup>	325	170	48 (44-52)
<i>C. TEP</i>				
1	10 <sup>-6</sup>	375	355	5
2	10 <sup>-5</sup>	810	730	11 (5-17)
4	10 <sup>-4</sup>	515	465	10 (5-15)
1	10 <sup>-4</sup> <sup>1</sup>	385	320	17
2	0.01	330	285	14 (10-18)
1	0.01 <sup>1</sup>	360	305	16
1	0.1	395	335	15
2	1.	325	195	40 (35-45)
2	1. <sup>1</sup>	320	205	36 (33-39)
2	10.	715	340	57 (49-65)
<i>D. ES</i>				
2	10. <sup>1</sup>	340	345	-2 (0-4)
2	20. <sup>1</sup>	400	290	28 (27-29)
<i>E. AS</i>				
1	0.01	340	340	0
2	0.1	585	555	5 (3-7)
1	0.1 <sup>1</sup>	225	230	-3
4	1.	545	435	20 (9-31)
2	1. <sup>1</sup>	255	255	0 (-4 to +3)

<sup>1</sup> These experiments in Warburg, others in capillary respirometer. Control values average higher in the capillary (525) than in the manometric (325) method; but % inhibition in comparable experiments is alike by both methods. The higher variation in the capillary is probably due to the much smaller samples used; the higher average perhaps to less damage. Some experiments at one concentration are given individually to show the similarity of inhibition despite variation in conditions. Values in ( ) indicate the range of inhibition, which is much less than the range of absolute values from brain to brain.

mm. Spontaneous electrical waves are first affected at 10 mM, which leads to a prompt and marked slowing to 2 to 3 a second, with no initial change in amplitude (fig. 1). Over about 10 minutes, amplitude falls greatly, with spindling recurring at 10-second intervals, but there is no further frequency change.

In the presence of caffeine, 1 mM DFP inhibits respiration during the second hour by 20 per cent; 5 mM, by 35 per cent (table 5 B). Even 0.1 mM markedly increases the after train of caffeine spikes. At 5 mM the caffeine spikes begin to be depressed in amplitude and frequency, and at 10 mM they are abolished by a 2-minute

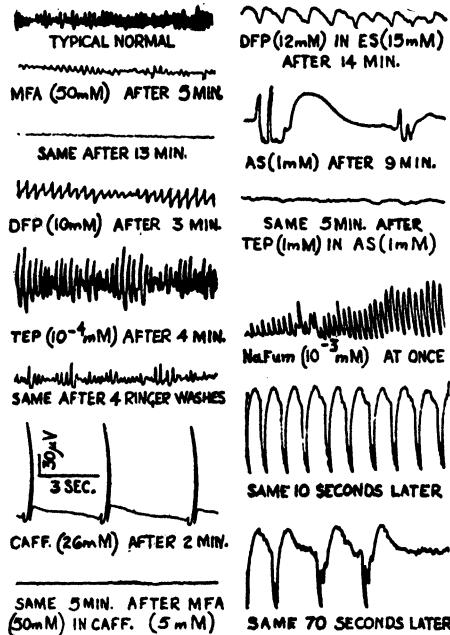


Fig. 1

soaking. All the DFP effects can also be stopped or reversed by washing within a few minutes of drug application.

**TEP.** Both electrical activity and respiration are affected by this agent at far lower concentrations than by the others.  $10^{-5}$  mM solutions depress respiration by 10 per cent, and even 0.1 mM depresses little more, by 15 per cent. One to 10 mM concentration cuts respiration to half (table 1 C). Three  $\times 10^{-4}$  mM inhibits ChE about 50 per cent. The spontaneous electric waves are first altered by  $10^{-4}$  mM TEP, which triples their amplitude without affecting frequency (fig. 1). No further action is obtained by increasing the concentration through the one to 10 mM range, but at 10 mM activity is promptly abolished. (In late spring to early fall frogs only a depression in amplitude appeared, beginning at  $10^{-4}$  mM and being complete at 10 mM.)

In the presence of caffeine (.5%), 1 mM TEP inhibits respiration by 15 per cent,

5 mm by 40 per cent (table 5 *B*). This is the TEP concentration range in which the effect on caffeine waves changes sharply, being increased in amplitude and frequency for several minutes by 1 mm solutions, depressed by stronger ones. Five mm TEP abolishes caffeine activity over 15 minutes, 10 mm, within a minute or two. Some, but incomplete, reversibility of these effects is obtained by prompt washing.

*Eserine Sulphate or Salicylate (ES).* ES at  $3 \times 10^{-8}$  mm inhibits ChE about 50 per cent. One to 5 mm ES slows the spontaneous rhythm to half frequency and at 10 mm doubles amplitude as well. Occasionally, regularly-repeated convulsive spikes appear. Similar findings with ES, but at 0.01 mm, and with ACh have been reported earlier (7). The ES action is reversed by washing at 30 to 60 minutes. Ten mm ES does not alter  $Q_{O_2}$ , but 20 mm produces a 30 to 40 per cent inhibition, persisting over both the 2- and the 4-hour measurement periods (tables 1 *D* and 2 *A*). A control with like concentrations of sodium sulphate or salicylate showed no inhibition. These data were especially needed for study of the combined action of ES and more irreversible anticholinesterases.

Several workers have shown that prior treatment with ES and related drugs will protect against DFP action (8, 9, 48; but not in the roach, 10), and recent evidence (34) is clear that ES can protect rats against several LD<sub>50</sub>'s of DFP without preventing the almost complete inhibition (98-99%) of ChE. Block of nerve conduction by DFP can similarly be prevented by ES, ChE in this case being also protected from inhibition (11).

DFP or TEP (each 6 mm) inhibits frog brain  $Q_{O_2}$  in the presence of (and after 2 hours pretreatment with) ES (20 mm) to a deeper level than in the absence of ES—65 per cent inhibition versus 40. ES does not, therefore, fully prevent the action of these drugs, although ChE must have been entirely inhibited by the ES; but the effects on  $Q_{O_2}$  are not additive. A more searching experiment consisted of exposing hemispheres to ES (12 mm) for 15 minutes, then to DFP (3 or 6 mm) in ES for 15 minutes, then washing 5 times in Ringer over a further 40 minutes. Their  $Q_{O_2}$ 's were compared with those of hemispheres similarly exposed to DFP alone or merely to Ringer. The results (table 2 *B*) show a clear protection by ES against DFP inhibition and a lesser protection against TEP. During the first 2 hours of measurement, 85 per cent of the DFP and 35 per cent of the TEP inhibition is prevented. A comparable experiment on sciatic nerves (6 nerves, about 120 mg. per Warburg vessel) gave like results (table 2 *B*). DFP (3 mm) alone gave 35 per cent inhibition during the first hour, ES-DFP gave no inhibition, thus protecting 100 per cent. On the electrical side, also, ES can protect against DFP abolition of spontaneous waves. DFP (10 mm) alone slows the waves and then cuts them down and it at once abolishes the slow ones present after ES (2 mm). But in the presence of 15 mm ES, the slow waves continue for half an hour with DFP.

In both DFP and ES experiments,  $Q_{O_2}$  values for the third and fourth hours were higher for the drugged brain or nerve than for the controls. The reasons for this late change in inhibition (destruction of drug, e.g. 12, preservation of more substrate, etc.) and in protection have not been explored.

*Atropine Sulphate (AS).* If the effects of these anticholinesterase drugs were due to ACh accumulation, atropine might be expected to prevent them. (There

is much disagreement as to the extent of prevention of ACh, DFP, and TEP action by AS, see 27 and review 53.) It was earlier reported (7) that ACh, like eserine, pro-

TABLE 2. ES AND DFP OR TEP ON BRAIN AND NERVE RESPIRATION

*A. Combined Inhibition: Brain*

TIME, MIN.	ADDITION, $Q_{O_2}$		INHIBITION, %	ADDITION, $Q_{O_2}$		INHIBITION, %	ADDITION, $Q_{O_2}$		INHIBITION, %
0-240	Ringer	ES <sup>1</sup>		Ringer	ES		Ringer	ES	
0-15	415	430		430	445		395	400	
60-120	300	180	40	320	235	27	305	200	35
120-240	Ringer	Ringer		DFP <sup>2</sup>	DFP		TEP <sup>3</sup>	TEP	
180-240	240			140	80		145	80	
% inhibition <sup>4</sup>		52		43	67		40	67	
% inhibition <sup>4</sup>				30			30		

*B. Protection by ES Pretreatment (see text)*

TISSUE	ES $Q_{O_2}$	R DFP (3mM) R $Q_{O_2}$	INHIBITION, % R $Q_{O_2}$	ES <sup>5</sup> DFP (3mM) R $Q_{O_2}$	INHIBITION, % R $Q_{O_2}$	R DFP (6mM) R $Q_{O_2}$	INHIBITION, % R $Q_{O_2}$	ES DFP (6mM) R $Q_{O_2}$	INHIBITION, % R $Q_{O_2}$	R TEP (5mM) R $Q_{O_2}$	INHIBITION, % R $Q_{O_2}$	ES TEP (5mM) R $Q_{O_2}$	INHIBITION, % R $Q_{O_2}$	
<i>First Hour</i>														
Brain	445	315	29	435	2	240	11	255	5					
	270													
	190													
Nerve	100	75	25	105	0					125	34	145		24
<i>Second Hour</i>														
Brain	375	300	20	350	7	120	40	205	0	75	57	110		37
	200													
	175													
Nerve	85	55	35	55	35									
<i>Third Hour<sup>6</sup></i>														
Brain	305	225	26	265	13	100	29	90	36	60	56	85		37
	140													
	135													
Nerve	75	65	12	50	33									

<sup>1</sup> ES = 20 mM in Ringer.   <sup>2</sup> DFP and TEP = 6 mM in Ringer.   <sup>3</sup> At 180-240 min.Compared to Ringer— $Q_{O_2}$  240.   <sup>4</sup> At 180-240 min. Compared to ES— $Q_{O_2}$  115.   <sup>5</sup> ES 12 mM throughout. Salicylate used with DFP, sulphate with TEP.   <sup>6</sup> Fourth hour with TEP.

ES present with DFP or TEP in all cases when present before.

duced increased electrical activity of the frog brain and that this was not altered by atropine. On the metabolic side, surprisingly little attention has been accorded ACh. A questionable increase in the  $Q_{O_2}$  of eserized frog brain by ACh addition was found by Lipton (reported in 13).

AS (1 mm), after 3 to 5 minutes soaking, may inhibit brain  $Q_{O_2}$  by 15 to 20 per cent (table 1 *E*), measured after an hour of equilibration and on small bits. Exposure to TEP (.01 mm, 5 minutes soak) subsequent to AS led to a greater inhibition of respiration (45%) than exposure to TEP alone (15%). With stronger TEP (1 mm), inhibition with or after AS (1 mm) could exceed 95 per cent (table 3). Results with DFP and AS were comparable; inhibition of respiration by DFP alone

TABLE 3. AS AND TEP OR DFP ON BRAIN RESPIRATION

TIME, MIN.	ADDITION, $Q_{O_2}$		INHIBITION, %	ADDITION, $Q_{O_2}$	INHIBITION, %	ADDITION, $Q_{O_2}$	INHIBITION, %
0-5 5-10	Ringer Ringer	Ringer AS 1 mm		Ringer TEP .01 mm		AS 1 mm TEP .01 mm	
60-90 60-90	680 510	520 410	24 19	570 440	16 14	390 300	43 41
0-5 5-10	Ringer Ringer			Ringer TEP 1 mm		AS 1 mm TEP 1 mm	
60-90	495			285	43	10-	98+
0-90	Ringer					Ringer	
30-90	255					265	
90-210	Ringer					AS 1 mm, TEP 1 mm	
90-150 150-210	305 220					225 105	26 52
0-120	Ringer			Ringer		AS 1 mm	
0-60 60-120	235 200			215 215		230 205	0 0
120-300	Ringer			DFP 3 mm		DFP 3 mm	
180-240 240-300	180 215			160 205	11 5	125 170	30 21

First two experiments in capillary respirometer, others in Warburg.

being under 10 per cent, by DFP after atropine, 25 per cent (table 3). On the electrical side, 1 to 10 mm AS slows the spontaneous rhythm by 30 to 50 per cent and progressively cuts amplitude to zero in 10 minutes. Convulsive spikes appear, however, and grow in size (to 250  $\mu$  V) while decreasing in frequency (to 1 in 6 sec.) over a 30-minute period (fig. 1). These spikes are not abolished within 5 to 10 minutes by amounts of DFP and TEP which abolish the usual spontaneous waves at once. Concentrations of AS which do not lead to spiking do not alter the electrical effects of TEP, over the range  $10^{-6}$  to 1 mm.

*Dicarboxylic Acids.* Fumarate, particularly, is able to counteract the inhibition of oxygen consumption and block of conduction induced in nerve by MFA (5). We have made comparable experiments on brain and with other inhibitors. Sodium fumarate (16 mM), present before addition of MFA (11 mM) from a side arm, prevents 75 per cent of the usual inhibition of  $Q_{O_2}$  produced by MFA alone (table 4).

TABLE 4. PROTECTION BY FUMARATE OF BRAIN RESPIRATION

TIME, MIN.	ADDITION, $Q_{O_2}$		INHIBITION, %	ADDITION, $Q_{O_2}$	INHIBITION, %
0-120	Ringer	Ringer		Fumarate 16 mM	
60-120	440	460		450	
120-240	Ringer	MFA 11 mM		MFA 11 mM	
120-180	375	290	23	360	4
180-240	340	205	40	305	10
0-60		Ringer		Fumarate	
0-60		295		310	
60-300		DFP 6 mM		DFP 6 mM	
60-120		220		270	
120-180		180		225	
180-240		160	(43) <sup>1</sup>	155	
240-300		110		105	(29) <sup>2</sup>
0-60		Ringer		Fumarate	
0-60		370		380	
60-300		TEP 1 mM		TEP 1 mM	
60-120		290		325	
120-180		250		265	
180-240		240	(40) <sup>1</sup>	265	
240-300		205		240	(34) <sup>2</sup>

<sup>1</sup> From experiment II A, run under parallel conditions.

<sup>2</sup> Calculated on basis of interpolated inhibition.

The fumarate itself perhaps increased respiration slightly, 2 to 3 per cent. In comparable experiments, fumarate (16 mM) gave a 30 per cent protection against the inhibition of oxygen consumption by DFP (6 mM), half this protection against TEP (1 mM) inhibition (table 4).

Attempts to explore the protective action of fumarate on the electrical effects of the experimental drugs were confounded by an entirely unexpected action of fumarate itself. (Fumarate was finally demonstrated to prevent the electrical changes, as well as most of the respiration inhibition, induced by MFA.) Even at

0.001 mm concentration, soaking for 3 minutes in Na fumarate leads to a dramatic change in the spontaneous brain potentials. The waves become progressively larger and slower until convulsive-type swings of 200  $\mu$ V at one per second are reached. The brain then becomes abruptly silent for minutes, when the whole sequence may repeat or stop for good (fig. 1). Similar effects, but at rather greater concentration, are produced by glutamate (1 mm),  $\alpha$  ketoglutarate (5 mm), oxaloacetate ( $10^{-2}$  mm), aspartate ( $10^{-2}$  mm), and succinate (1-10 mm). Valine, pyruvate and glucose, on the contrary, are entirely inert. The action appears to be associated with the presence of the 4 to 5 carbon dicarboxylic skeleton. These results were obtained consistently in many experiments during the winter of 1948 at Chicago. Attempts to repeat them the following summer were negative, no effect of these substrates being observed. Several have again been obtained here this winter (but fumarate only at 10 mm or above) plus the further finding that when a brain has become silent after, say, fumarate, it can respond well to, say, aspartic and after this will respond again to fumarate. We cannot now account either for the phenomenon or its variability. Sex and temperature at which the frogs are kept are immaterial; different batches of chemicals, with or without recrystallization, have behaved alike. If impurities are involved, they must be extremely potent. The marked variation in blood sugar of frogs, from 75 mg.% in summer to 200 mg.% in winter (11a) deserves note; as also a seasonal difference in drug sensitivity (12a, 13a).

**Caffeine.** Since this drug was used (as alkaloid) regularly in the electrical experiments, a few tests were run on its influence on respiration. Exposure for 2 to 3 minutes to 0.5 per cent caffeine (26 mm) increases oxygen consumption by a third, as it also increases the brain's electrical activity; and even greater increases, up to doubling, are obtained on long exposure to 0.1 or 0.25 per cent caffeine. Similar long exposure, one or two hours, to 0.5 per cent caffeine inhibits respiration 15 per cent, and also abolishes all electrical activity (table 5 A). DFP, added with 0.5 per cent caffeine, does not increase the caffeine inhibition when in a concentration of 1 mm, which increases caffeine waves, but does inhibit further (doubles the inhibition) in a concentration of 5 mm, which depresses caffeine waves. MFA, 12 mM, or TEP, 1 mM, does not increase the caffeine inhibition, but 5 mM TEP, which depresses caffeine waves, does (table 5 B).

**Nerve.** A few experiments on frog sciatic nerve respiration (by K. S. Crippen) yielded results similar to those on brain. One to 4 runs each were made in duplicate with each agent, compared to Ringer controls, on 5-mg. pieces of nerve in the capillary respirometer. Nerves were soaked for 1 to 2 hours (all solutions at pH 6.9-7.3) before mounting and then followed for 60 to 90 minutes. At 10 mm concentration, neither fumarate or succinate increased oxygen consumption, nor did 30 mM malonate inhibit. TEP,  $10^{-3}$  mm, gave a 40 per cent inhibition; DFP, 5 to 20 mM, a 60 per cent inhibition; and ES, 20 to 40 mM, a 25 per cent inhibition. Our findings are summarized in table 6.

#### DISCUSSION

Evidence has been offered that the fluoroacetates specifically inhibit acetate oxidation, presumably due to competitive inhibition by the halogen compound of appro-

TABLE 5. CAFFEINE ON BRAIN RESPIRATION  
*A. Alone*

TIME, MIN.	ADDITION, $\text{Q}_{\text{O}_2}$		INCREASE, %
0-10	Ringer	Caffeine 0.5%	
0-60	565	585	3
0-60	570	755	24
0-60	585	840	41
0-240	Ringer	Caffeine 0.5%	
0-60	330	235	-28
120-180	170	145	-15
180-240	155	130	-17
0-120	Ringer	Caffeine 0.25%	
0-60	340	430	26
60-120	265	395	49
0-180	Ringer	Caffeine 0.1%	
0-60	510	575	13
60-120	440	455	4
120-180	360	270	-27
0-60	200	375	88
60-120	100	275	175

*B. With Other Drugs*

TIME, MIN.	ADDITION, $\text{Q}_{\text{O}_2}$		INHIBITION, %		
0-240	Ringer	Caffeine 0.5%			
0-30	650	590	9		
60-120	360	300	17		
120-240	Ringer	DFP 1 mM	DFP 5 mM		
120-180	365	320	230	12	37
180-240	260	200	180	23	31
0-120	Ringer	Caffeine + MFA 12 mM			
60-120	560	455		20	
0-120	Ringer	Caffeine + TEP 1 mM			
60-120	635	505		21	
60-120	415	360		13	
0-120	Ringer	Caffeine + TEP 5 mM			
60-120	375	215		43	

TABLE 6. SUMMARY

NO. ELECT. EXPER.		DRUG	CONC. (mM)	ACTION ON FROG BRAIN (NORMAL LEVEL = 1.0)							
				Electrical activity				QO <sub>2</sub>		Ch. E.	
				Spont. rhythm		Caff. waves		Brain		Nerve	Brain
No caff.	caff.			Ampl.	Freq.	Ampl.	Freq.	Normal	Caff.		
9	9	NaFA	50	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
24	MFA		5	1	1	1	1	0.9		0.2	
			12	0.5	1	1 +→1	1 +→1	0.5	0.8		0.5
			25	0.5	1	0.3→0	0.3→0				
			50	0.5	1	0	0				
			100	0	0	0	0	0.2			
10	DFP	-1" data-rs="5">	10 <sup>-1</sup>	1	1	1 +→1	1	1.0			
			0.3	1	1	1 +→1	1	1.0			
			1	1	1	1 +→1	1 <sup>2</sup>	0.9	0.8		0.5
			5	1	1	0.5	0.6				
			10	1→0.2	0.5	0	0	0.5	0.4		
47	TEP	-6" data-rs="9">	10 <sup>-6</sup>	1	1	1	1	0.9			
			10 <sup>-4</sup>	3	1	1	1	0.8			
			3 × 10 <sup>-4</sup>	3	1	1	1	0.8			
			10 <sup>-3</sup>	3	1	1	1	0.8			
			10 <sup>-1</sup>	3	1	1	1	0.8			
			1	3	1	1+	1+	0.6	0.8		
			5	3	1	0.5→0	0.6→0	0.6	0.6		
			10	0	0	0	0	0.5			
			3 × 10 <sup>-3</sup>	1	1						
23	ES		1	1	0.5			1.0			
			1	1	0.5			1.0			
			5	1	0.5			1.0			
			10	2	0.5			1.0			
			20					0.6	0.8		
13			1	1→0	0.5 <sup>3</sup>						
			10	0	0						
7	ACh	-3" data-rs="3">	10 <sup>-3</sup>	1	1	5	0.5				
			1	1	1	1	0.5				
			10	0.2→0	1 <sup>4</sup>	0	0				
10			5	1	1			2			
			25	1 +→0	1 +→0 <sup>5</sup>			1.3→.8 <sup>1</sup>			

<sup>1</sup> DFP or TEP depressed QO<sub>2</sub> when waves depressed. <sup>2</sup> caffeine train increased. <sup>3</sup> spikes not abolished by DFP or TEP. <sup>4</sup> spikes appear. <sup>5</sup> spikes and train appear.

priate dimensions to fit the active enzyme (6, 14). Other work (15, 16, 16a) has emphasized an action on the oxidative decarboxylation of pyruvic acid and has thrown

doubt on the competitive nature of the inhibition. Our finding on brain, as well as comparable ones on nerve (5), brain brei (3), and muscle (4) that MFA is far more potent than NaFA, also speaks against a precise steric interaction of the fluoroacetate radicle with the enzyme. All workers (14, 16, 17, 18) offer evidence of interference with the formation or oxidation of succinate or citrate (18a, 19a) and most believe that the tricarboxylic cycle is interfered with, whether decarboxylation or a splitting of active acetate (14) occurs at the oxidative steps. The present findings on brain and comparable ones on nerve (5), that MFA inhibition of respiration and of function can be overcome by several tricarboxylic cycle intermediates, notably fumarate, also strongly suggest an action on the Krebs cycle. We would particularly suspect the step from alpha ketoglutaric to succinic except for the finding on muscle (17) that MFA inhibits succinic but not alpha ketoglutaric oxidation. Our results, then, favor the existence and functional importance in brain (and nerve) of the Krebs cycle. No one has suggested that MFA affects ChE, *in vitro* evidence (6) being to the contrary except for an experiment here in which 20 mM MFA inhibited one-third of the ChE activity of frog brain on a 30-minute incubation *in vitro*.

DFP, TEP, and HTP, in contrast to MFA, are widely recognized as ChE inhibitors, and fluorophosphonates have been found not to inhibit a variety of other enzyme systems *in vitro* (19). It is commonly assumed, therefore, that these substances produce functional changes by their action on the ACh system. MFA, however, was likewise found (6) inactive on 18 purified enzymes, including the very ones that are clearly affected *in vivo*; and comparable cases of insensitivity of purified systems are well known (20). DFP, TEP and eserine have now been shown to inhibit oxidations of frog nerve, or rat brain brei, and, in the present experiments, of frog brain slices, in concentrations comparable to those which are physiologically active or which inhibit ChE (e.g. eserine on nerve, 21-24). DFP and TEP, at 0.1 mM, were also observed in other experiments to inhibit the growth of *lactobacillus arabinosis* (C. Haber). Furthermore, although some work (25-28, 30-32) favors the conclusion that symptoms and death produced by anticholinesterases run parallel with the degree of inhibition of brain ChE, (although the level of the ChE at which death appeared varied in different reports between 0 [30, 36] and 95% [35] normal), other recent studies (32-34) trend in the other direction. High ChE activity, over 80 per cent normal, may be present in the brains of rats dying from ES (35) and low ChE activity (15%), in the brains of survivors (34, see also 32). Further, rats pre-treated with ES and surviving 2 to 4 times the LD<sub>50</sub> of DFP, consistently show less than 2 per cent of the normal ChE activity of cortex or caudate or, with larger ES and DFP doses, followed hour by hour, become symptom-free while the ChE is still falling (at 10-15% normal activity). Accumulation of high ACh concentrations in the brain support the evidence that ChE is inhibited *in vivo* (34). Comparable results on ChE are reported for monkeys (36); see also (37). It has even been found that when ChE is fully inactivated to ACh by ES its activity on another substrate (chloracetic ethyl ester) is unimpaired (38). The literature is conflicting enough, even on whether true ChE is as sensitive as pseudo ChE to these drugs (36, 39) or not (38, 27, 40, 36, 31, 19) so that a summary is dangerous; but table 7 cannot be far off in order of magnitude.

From table 7 the following rough statements can be made. All nerves tested

(invertebrate giants, mammalian sympathetic, frog and fish somatic) block within an hour when exposed to any of the antiesterase drugs in the concentration range of 10 to 20 mM. (DFP on roach giant is 60 mM.) TEP is somewhat more active in general, ES and DFP change positions from case to case. In DFP-blocked nerves, the ChE is found to about 80 per cent inhibited by 3 workers, 92 or 95 per cent in 2 other papers; in nerves conducting fully, the ChE inhibition is reported by 3 groups to be 100, 98, and 93 per cent inhibited. The concentration of a given drug in a given species required to block nerve conduction is 1,000- to 100,000-fold greater than that needed to alter central nervous function. Brain ChE of mammals is 50 per cent inhibited *in vitro* by TEP or DFP at a concentration range centering on  $10^{-5}$  mM (but some reports put DFP concentrations at  $10^{-3}$  or higher); by ES at  $10^{-3}$ . Frog brain is less than one-hundredth as sensitive to ES as is mammalian brain (50% inhibition at  $10^{-3}$  and  $10^{-5}$ , respectively) in comparable experiments; roach cord is as sensitive or more so to ES (50% inhibition at  $10^{-5}$  mM) and less sensitive to TEP or DFP (50% inhibition at  $5 \times 10^{-4}$  and  $5 \times 10^{-2}$ , respectively) than is mammalian brain. The *in vitro* drug concentration required for altered central nervous function and for 90 per cent ChE inhibition is often similar but may differ by a power of 4 (ES on roach cord). *In vivo* administration to mammals indicates that TEP is more lethal than DFP ( $LD_{50}$ : TEP, about 0.002 mM/K; DFP about 0.02) and there is the same disagreement as for nerve as to whether death parallels ChE inhibition (50-100% inhibition reported in fatalities at  $LD_{50}$ ; 75% to over 90% in survivors—even at  $LD_0$  with DFP). Since lethality may depend on particular sites of action, central or peripheral, and drug penetration may complicate *in vivo* studies (34, 35), such disagreements might be expected. It seems clear, none the less, that there is inadequate justification for attributing the neural effects of the 'anticholinesterase' drugs primarily to their inhibition of ChE and an attendant accumulation of ACh.

It is, in fact, unlikely that all these drugs act even on the same link in the metabolic chain, at least in the same neurones; for if so their effects should be essentially identical and other agents should alter these in similar fashion. Yet DFP first slows the normal electrical waves without changing their amplitude, while TEP mainly increases and MFA decreases their amplitude without altering their rate. Caffeine waves are unchanged in frequency, except for a transient speeding up by MFA, while all 3 drugs increase their amplitude, at least transiently, at appropriate concentrations. Caffeine, itself reported to inhibit ChE (54, 60, and, at higher concentrations, succinic dehydrogenase, 55), produces entirely different electrical effects. These various drugs do not, therefore, act alike, as if each inhibited ChE specifically; rather all produce somewhat unique functional changes, as if each tangles with the metabolic belt in its own way. This is further indicated by the inability of TEP and MFA or of TEP and ACh, each present in half that concentration needed to stop electrical activity, to sum their effects. In such mixtures, activity continues. Further, ES protects respiration and electrical activity against DFP doses that would normally stop brain waves at once; while AS does not protect against DFP (both results occur in mammals; 49, 57, 53 and references)—it actually enhances the depression of respiration by DFP and TEP—nor do these drugs prevent the electrical effects of AS itself. There is, thus, no evidence for a common action on the ACh-ChE system.

It is noteworthy, though, that these drugs (except ES), with or without caffeine,

TABLE 7. SUMMARY OF LITERATURE

## A. Nerve

ANIMAL	NERVE	DRUG	IN VIVO		IN VITRO		
			Conc. mm/K	LD	Conc. (mm)	% ChE inhib. in nerve	Conc. (mM)
Roach	Giant fiber	HTP	.01	50 (26)			7 (25)
		DFP	.03	50 (26)			60 (25)
Squid	Fin	DFP			13	80 (41)	13 (41)
Earth-worm	Giant fiber	DFP					25 (21)
Cat	Cervical symp.	DFP			20	80 (23)	20 (23)
	Tibial	ES DFP					20 (23) 20 (22)
Skate	Optic	DFP					15 (43)
Frog	Sciatic	DFP	11.		98 (22)	(22)	No block
				3	100 (44)	15 (44)	No block
		E(S)		3	93 (24) <sup>1</sup>	3 (24)	No block
						20 (21, 44, 23, 22)	Block in 20 min.
						20 (23, 22)	Block in 20 min.
						50 (21)	Block in 70 min.

## B. Brain

ANIMAL	DRUG	IN VIVO <sup>2</sup>				IN VITRO			
		Conc. mm/K	LD	% inhib. brain ChE		Conc. mm	%	ChE inhib. in brain	Conc. mm
				Survivors	Fatalities				
Roach <sup>4</sup>	HTP	.01	50 (26)			2 × 10 <sup>-4</sup>	50 (26)	5 × 10 <sup>-4</sup> (10)	Synapse change
	DFP	.03	50 (26)			10 <sup>-2</sup>	50 (26)	.6 (10)	Synapse change
	ES					10 <sup>-5</sup>	50 (26)	.2 (10)	Synapse block
Frog	ES					10 <sup>-3</sup>	50 (50)		
Man	DFP					10 <sup>-8</sup>	50 (36)		
Monkey	DFP	.001 .0005	50 (40)	100 (36)	100 (36)	3 × 10 <sup>-3</sup>	50 (36)		

TABLE 7—Continued

ANIMAL	DRUG	IN VIVO <sup>2</sup>				IN VITRO			
		Conc. <sup>3</sup> mg./K.	LD <sub>50</sub>	% inhib. brain ChE Survivors	% inhib. brain ChE Fatalities	Conc. mM	% ChE inhib. in brain	Conc. mM	Physiol. effect
Rabbit	DFP	.02	50(40)	75(30)	75(30)	100(30)			
Dog	HTP	.002 .01	50(27) 0(27) <sup>5</sup>	96(27)	60(27) <sup>6</sup>				
	DFP ES	.02	50(40)			10 <sup>-1</sup> 10 <sup>-5</sup>	50(37) 50(56)		
Cat	HTP	.001 .004						(52) (52)	N-Mine NM block
	DFP	.01-.02	50(48, 40)					.02 .02-.07 (48)	EEG change NM block
Mouse	HTP	.01 .006	50(27) 0(27)	33(27) <sup>7</sup>					
	TEP	.003	50(32)						
Rat	DFP	.02	50(40)						
	HTP	.005 .02	50(50) 50( 8)		50(50)	10 <sup>-5</sup>	50(50)		
	TEP	.002	50(32)	33(32)	72(32)	4-6 × 10 <sup>-6</sup>	50(28, 32)		
	DFP	.004 .01	50(28) 50(51, 37, 40)			6 × 10 <sup>-5</sup>	50(50)		
	ES	.005 .007	50(28) 0(37)	90(37) <sup>8</sup>		4 × 10 <sup>-4</sup>	50(28)		
	PS <sup>9</sup>	.006	100(35)		30(35)	10 <sup>-4</sup>	60(35)		
					5(35)	3 × 10 <sup>-2</sup>	50(37)		
						10 <sup>-3</sup>	50(31)		
						10 <sup>-4</sup>	40(35)		

<sup>1</sup> In various papers, these workers have reported that nerve block occurs when ChE inhibition is greater than the critical value of: 80% (42), 92% (23, 30), or 95% (24).

<sup>2</sup> Other findings: For all four antiesterases, brain ChE is 50% inhibited at the LD<sub>50</sub> (28); but (33) brain ChE is inhibited alike in fatalities and survivors with DFP or ES. DFP symptoms parallel the brain ChE inhibition (51); but symptoms begin at 20-30% ChE inhibition (31).

<sup>3</sup> These figures are calculated from mg./K. Route of injection was not alike in all. <sup>4</sup> Ganglionated cord. <sup>5</sup> Protected by atropine. <sup>6</sup> 96% ChE inhibition at 3 hours; 65% at 20 hours.

<sup>6</sup> It is reported that HTP death occurs at 80-90% ChE inhibition (27). <sup>7</sup> Killed at 15 min. At 2 hours, inhibition is 26%. It is reported (32) that at LD<sub>50</sub> brain ChE inhibition with HTP averages 50% (range 25-75). <sup>8</sup> At 5 to 24 hours. At 10 days, 55% inhibition. Other workers (33) report the same ACh concentration in brains of DFP survivors and fatalities. <sup>9</sup> PS = Prostigmine methyl sulphate.

inhibit the over-all oxygen consumption by 20 to 50 per cent at the concentration, in each case, which provokes functional changes—from  $10^{-4}$  mM in the case of TEP to 12 mM in the case of MFA; and, where tested, they inhibit oxygen consumption by 40 to 50 per cent at the concentration which abolishes electrical activity. Further, from concentrations of DFP and TEP which augment caffeine waves to those which depress, there is a sharp decrease in respiration. The tempting possibility that ChE is involved in normal respiration (e.g. in relation to the choline of lecithin) is excluded by the ability of ES to inhibit ChE without altering oxygen consumption. Others (10, 33, 9, 45, 46) have also noted different effects of different 'antiesterases,' even on the EEG (47, 59), and suspected actions other than ChE inhibition (21, 58). The ratios of concentrations for the various 'antiesterases' for critical action on various systems or species are so widely different as also to indicate other actions of these drugs.

#### SUMMARY

The isolated frog brain (and nerve) has been used to study the influence of a number of substances on function, indicated by normal and caffeine-induced electrical waves, and on metabolism, indicated by oxygen consumption and cholinesterase activity. The fluoroacetates (NaFA, MFA), inhibitors of carbohydrate metabolism, and the fluorophosphates (DFP), polyphosphate esters (TEP), eserine, and atropine, recognized as acting on the ACh-ChE system, affect function and metabolism in comparable ways, not in different ones. At concentrations in each case (except ES) which alter electrical activity, respiration is inhibited 20 to 50 per cent (about 50% when electrical waves are abolished) but ChE inhibition may be absent or nearly complete. Results with these drugs, caffeine, and ACh are summarized in table 6.

There is no summation of the actions of TEP and MFA or of TEP and ACh. Eserine can largely or fully protect brain and nerve from DFP depression of both function and respiration. Atropine does not protect against TEP or DFP nor is its own action prevented by them. Caffeine in small doses increases electrical activity and oxygen consumption, in larger doses depresses both. Inhibitors combined with caffeine similarly increase or decrease function and respiration in parallel at appropriate concentrations.

Fumarate can protect against MFA action and it, with several other dicarboxylic acids at low concentration—glutamic,  $\alpha$ -ketoglutaric, oxaloacetic, aspartic, and succinic (valine, pyruvate, and glucose are inert)—is able to initiate large convulsive potentials in the isolated brain. These effects have been variable.

Our evidence favors a significant role of the tricarboxylic cycle in brain metabolism, one related to the maintenance of function. The present results on frog brain and nerve, as well as those in the literature on rats and other animals, indicate that DFP and TEP disturb function by mechanisms independent of the simple inhibition of ChE. An interference with oxidative metabolism is indicated.

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# RESPIRATORY FUNCTION AND BLOOD FLOW IN THE BRONCHIAL ARTERY AFTER LIGATION OF THE PULMONARY ARTERY

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IT HAS long been known that the blood arriving by way of the bronchial arteries is in itself sufficient to support the life of the lung, provided that there is not an excessive back pressure in the pulmonary veins (1-3). Schlaepfer (3, 4) has demonstrated that such a lung, deprived of its pulmonary arterial supply, suffers little histological change, even during a two-year interval. It merely becomes reduced to three-fourths or two-thirds of its usual size and there is slight fibrosis about the bronchovascular rays and in the walls of the alveoli. In these lungs there has been observed grossly an enlargement of the bronchial arterial channels, as well as the development of collaterals from the esophageal and other vessels of the mediastinum (2, 3, 5, 6).

That blood brought to the lungs by way of such arterial channels can enter the capillaries of the alveoli and perform a respiratory function is implied by the existence of instances of pulmonary atresia in man, where branches of the descending aorta constitute the sole arterial supply of the lungs and maintain life for many years (7, 8). Actual indirect measurements of the volume of blood arriving by way of collateral channels in cases of pulmonic stenosis have recently been made by Bing and his associates (9). Anatomical studies of bronchovascular casts of the lungs also have demonstrated the enormous collateral bronchial arterial circulation that may exist in congenital pulmonic stenosis (10) and in chronic bronchiectasis (11). The possible rôle of this systemic collateral in shunting blood from points of anastomosis within diseased tissue to healthy pulmonary parenchyma has been discussed (11). In the intact lung of the dog and of man precapillary anastomoses between the bronchial and pulmonary circulations do not normally exist (12), but blood may reach the capillaries of the alveoli from the bronchial vessels if the pressure in the pulmonary artery is reduced (13).

The present experiments were designed to test, in the dog, in what measure a lung whose main pulmonary artery has been ligated can carry on the function of respiration, and to determine whether such a respiratory function may increase with time. Anatomical studies of the lungs of these animals are reported elsewhere (6).

## METHODS

The posterior operative approach to the left pulmonary artery through the fifth interspace was used. This was advantageous in comparison with the anterior ap-

Received for publication January 7, 1949.

<sup>1</sup> Performed under a contract with the Office of Naval Research as Project Noori 44, Task Order XI.

proach in that a longer segment of the artery could be visualized and isolated for ligation, and retraction of the pulmonary vein was less difficult.

For the bronchspirometric studies a cannula was constructed somewhat on the pattern of the instrument previously described by Van Allen and Lindskog (14). This provided a convenient means of cannulating the right and left halves of the respiratory tree separately. The new cannula consists of two parts: A narrow tube (the left bronchial cannula) 51.5 cm. long and 7.5 mm. in external diameter, which fits within another tube (the tracheal cannula) 31 cm. long and 1.5 mm. in diameter. When the latter is introduced into the trachea, the distal end of the bronchial cannula can be placed under direct vision into the left main bronchus whose respiratory exchange is thus isolated. The short tracheal tube carries the exchange of the right lung, which is brought out by means of a side arm 7 mm. in internal diameter. The tracheal tube is surrounded by a collapsible rubber cuff to insure an airtight intra-

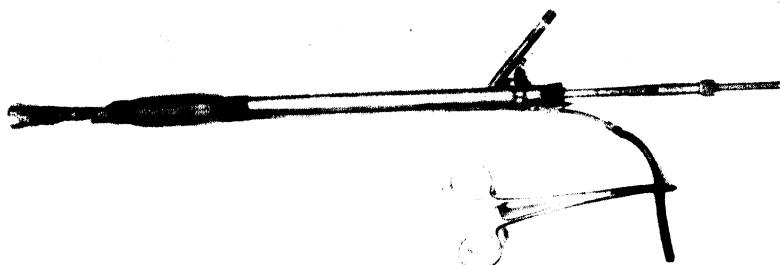


Fig. 1. BRONCHOSPIROMETRIC CANNULA. The Van Allen bronchial unit is shown held in place within the tracheal cannula. In practice the rubber stopper is not inserted until the bronchial unit has been fixed in the left main bronchus. The screw thread, manipulation of which spreads the rubber covered petals of the bronchial extremity, is at the right. The side arm connecting to the lumen of the outer, tracheal, unit projects obliquely upwards. The bag that assures a tight tracheal seal has been inflated. In use, a pressure bulb and mercury manometer would be attached to the rubber tube that is shown clamped.

tracheal seal. The inner bronchial element is held in place with a rubber stopper coated with vinylite after it is seated, to prevent leakage of gases. The bronchial element has a larger lumen than Van Allen's original cannula, to reduce the factor of obstruction. This element consists of two closely fitted tubes which are joined at the external end by a screw thread (figs. 1 and 2). By use of this thread the inner ends of the tubes can be separated or brought together. The outer sleeve has at its bronchial extremity four hinged petals, and the inner ends in a cone. When the inner tube is unscrewed the cone wedges the petals apart. These petals are covered with a rubber membrane to make an airtight seal with the wall of the bronchus.

The bronchspirometric test was performed after the dogs were anesthetized with sodium pentobarbital, 35 mg/kg. intravenously. Under direct vision the instrument was passed through the larynx until both tubes extended well into the trachea. A bronchoscopic light carrier was then introduced into the inner tube and the instrument was further advanced until the carina was visualized. The inner tube was made to enter the left main bronchus. The cannula was now fixed in place

by screwing the knob counter-clockwise, thereby opening the petals on the end. The rubber stopper was placed around the smaller tube, fitted snugly into the larger tube, and sealed off with vinylite. The tracheal bag was inflated and kept at approximately 100 mm. of mercury under manometric control.

Inspection of the cannula *in situ* at necropsy in a series of dogs demonstrated the utility of the instrument and the airtight seal at the left stem bronchus. To test for leaks around the inner end of the left bronchial cannula in living animals, a 'smoke test' was found useful. Cigarette smoke was blown into the left lung through the inner tube. Its failure to appear on the right side and maintenance of positive pressure after a few respirations was taken as evidence against the existence of a leak. When the absence of leaks was demonstrated each lung was connected to a recording spirometer. Any lag, inspiratory or expiratory, of one spirometer behind the other indicated partial obstruction and necessitated readjustment of the cannula.

Fig. 2. CLOSE-UP OF THE VAN ALLEN BRONCHIAL CANNULA IN OBLIQUE VIEW. It shows the petals covered with rubber. Retraction secured by manipulating the screw thread shown in fig. 1, of the pyramidal tip of the inner element spreads the petals and assures a tight connection with the lumen of the bronchus.



Two Benedict-Roth spirometers were used, one for each lung, employing a closed-circuit with flutter valves and soda lime carbon dioxide absorber between expiratory valve and spirometer. Both spirometers recorded simultaneously by means of inked pens on the same drum.

Blood specimens were drawn in oiled syringes containing a minute quantity of heparin powder, mercury sealed and capped, and chilled immediately in water containing ice. Analyses of carbon dioxide and oxygen content and carbon monoxide capacity were determined manometrically according to the standard methods in Peters and Van Slyke (15). Gas samples were collected over mercury and analyzed in the Henderson-Haldane apparatus.

#### PROCEDURE

The oxygen uptake of the left lung and the oxygen content of the arterial blood were measured under the following conditions:

- I. *Both lungs breathing air.*
- II. *Left lung breathing oxygen, right lung breathing air.*

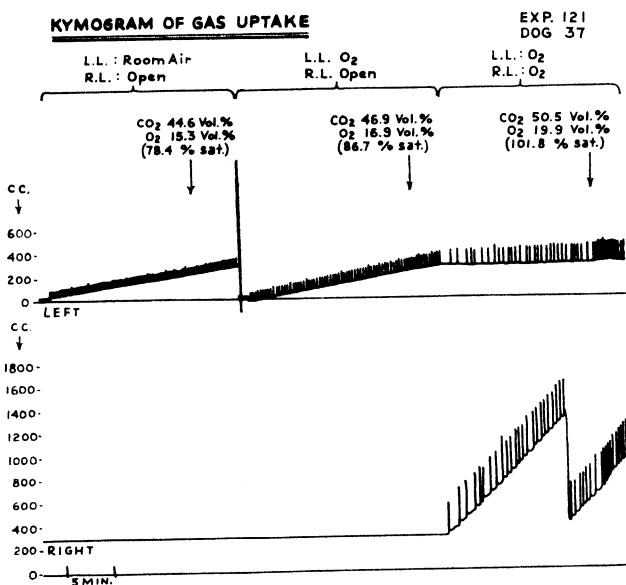


Fig. 3. A. UPPER GRAPH: Left lung. B. LOWER GRAPH: Right lung. Phase I at the left, phase II in center and phase III at right.

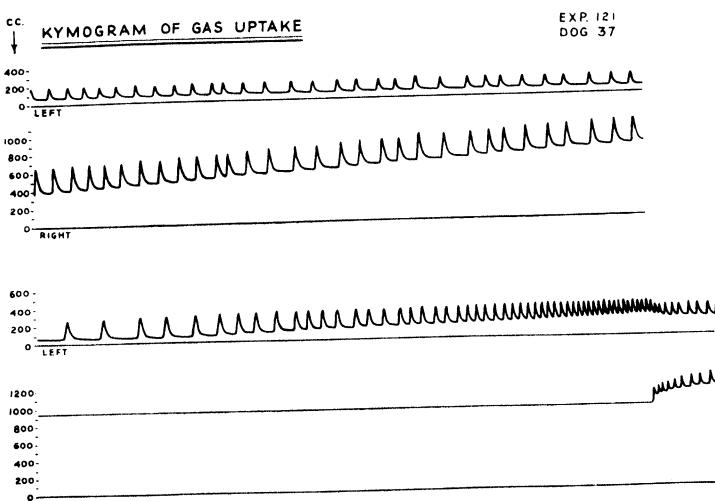


Fig. 4. A. UPPER PAIR OF GRAPHS: gas transfer with both lungs breathing oxygen. B. Lower pair of graphs: gas absorption of left lung, while the right lung is cut off. At the end of this run oxygen is again admitted to the right lung. Five-minute runs. Kymograph at fast speed.

III. Both lungs breathing oxygen.

IV. Left lung breathing oxygen, right lung blocked off.

The conditions of the experiment can best be analyzed by reference to a specific example (figs. 3 and 4):

I. With cannula in place, the left lung is connected to a spirometer containing a measured volume of room air, between 750 and 1,000 cc. The right lung breathes from the room. The systemic arterial blood arriving in the left lung via the bronchial arteries has a relatively low oxygen content (15.3 vol. %) as sampled at the femoral artery. The range of 10 determinations in 6 dogs was 8.9 to 17.1 volumes per cent, averaging 13.8 volumes per cent. The mean oxygen saturation in 7 determinations was 62.8 per cent. The low arterial oxygen content under these conditions is thought to be associated with respiratory depression and hydremia (5) and possibly with foci of pulmonary atelectasis resulting from the anesthesia together with the supine position of the animal. A considerable decrease in the gas volume of the spirometer takes place during the 20 minutes of the experiment. This decrease is due in part to oxygen consumption, but also in part to the transfer of nitrogen from the spirometer as its tension rises<sup>2</sup>.

II. When the air in the left spirometer is replaced with oxygen, respirations become slower and deeper. At the same time there is a more rapid absorption of oxygen (14.8 cc/min.) commensurate with a steeper oxygen tension gradient, and the arterial blood oxygen content in the present instance rises to 16.9 volumes per cent. The mean oxygen content rose 1.9 volumes per cent and the mean O<sub>2</sub> saturation from 62.8 to 81.6 per cent in the same animal reported in *phase I* of the experiment. Under these circumstances the decrease in the gas content of the spirometer is due solely to absorption of oxygen. Actually more oxygen is absorbed than is indicated by loss in volume, since diffusion of nitrogen now takes place from pulmonary capillaries into the spirometer in amounts usually ranging between 100 and 200 cc. in 20 minutes. (The average of 20 determinations was 156 cc./20 min.)

III. With the left lung still breathing oxygen, the right lung is now supplied with almost pure oxygen from its spirometer. Under these circumstances, the absorption from the left spirometer falls strikingly. The respiratory rate usually decreases, and the depth of respirations increases. The blood now has an even greater oxygen content than can be accounted for by the hemoglobin content as determined by the carbon monoxide capacity. In the present instance the oxygen content during *phase III* was 19.9 volumes per cent and the carbon monoxide capacity was 19.5 volumes per cent. In 42 determinations the oxygen saturation in *phase III* was less than 100 per cent in only 6 instances. This high oxygen content is to be expected from the absorption coefficient of the plasma. The plasma alone will dissolve as much as 2 volumes per cent of oxygen when in equilibrium with tensions of oxygen in excess of 700 mm. Hg (16).

The small uptake of oxygen when the bronchial arterial blood reaches the alveoli of the left lung results from the fact that it is already practically in equilibrium with the alveolar tension, having been exposed to an equivalent tension on the right side.

<sup>2</sup> The composition of the gases at the end of 20 minutes was not determined in this particular experiment, but in another experiment under similar conditions (exper. 123, dog 9) the analysis showed 13.1 per cent oxygen and 86.9 per cent nitrogen at the end of this period. The total volume of gases absorbed by the left lung during the 20 minutes was 230 cc., but referring to the composition of the 1195 cc. of gases in the spirometer at the beginning of the experiment, namely oxygen 20.0 per cent, carbon dioxide 0.1 per cent, and nitrogen 79.0 per cent, it is apparent that only 124 cc. of oxygen had been absorbed while 105 cc. of nitrogen were being absorbed at the same time. Thus 53.9 per cent of the gas absorption calculated from the spirometer record represented oxygen. These calculations take into account the dead space in the spirometer system.

Some reduction in saturation occurs from admixture of desaturated venous blood which enters the pulmonary veins, or the left side of the heart via Thebesian channels. We have repeatedly observed, in man, sizeable branches of the pulmonary vein which drain the posterior mediastinum. Some desaturated blood must also come from the walls of the bronchi and supporting structures of the lung.

IV. In the last section of the experiment, after the control interval of 5 minutes with both sides breathing oxygen, and while the kymograph is moving at fast speed (fig. 4A), the oxygen supply to the right lung is cut off for 5 minutes by clamping the rubber tubing inlet (fig. 4B). The oxygen content of the blood in the bronchial artery falls rapidly, approaching that of the mixed venous blood whose oxygen content is rapidly diminishing. The conditions of maximal absorption of oxygen by the blood actually perfusing the alveolar capillaries of the left lung now obtain. Inspection of the kymogram (fig. 2) reveals that the respirations at once become deeper and that their rate increases, particularly toward the end of the 5-minute interval. It is also obvious that the tracing of the gas absorption now is a curve with an increasingly steep slope, while the tracings during the previous phases of the procedure were linear. It will be noted that during the previous phase of the procedure the animals have had time to approach nitrogen equilibrium with the gases in the spirometer. Thus, during the 5 minutes of *phase IV*, under the influence of the steep gradient between the high tension of O<sub>2</sub> in the left spirometer and the deoxygenated blood, the absorption curve represents uptake of oxygen unmodified by significant nitrogen transfer.

The left lung becomes overexpanded as the right becomes increasingly atelectatic as indicated by the drop in the resting level of the left spirometer when the right lung is allowed to reexpand. Obviously the measurement of the oxygen absorbed must be referred to the resting level of the left spirometer after reexpansion of the right lung. Reexpansion occurs within the first few breaths after unclamping the right inlet tube. The right lung, however, may not reexpand quite to its former volume, introducing an error into the calculation of oxygen absorption by the left lung. In the present instance the oxygen absorbed from the left spirometer was 28.6 cc/minute.

Towards the end of the 5-minute interval the animals as a rule become severely dyspneic, or even apneic in the case of those with the more recent ligations. The bronchial circulation of the left lung is insufficient to support life for more than a few minutes, even 21 months after ligation of the pulmonary artery.

*Oxygen Absorption of the Lung in Relation to Time After Ligation of the Pulmonary Artery.* When the oxygen supply of the intact right lung is interrupted (as in *phase IV* of the experiment just described), the oxygen absorption of the left serves as a rough measure of the blood flowing through that lung. Analysis of the data, table 1 and figure 5, shows an upward trend as the time after ligation of the left pulmonary artery increases. Sixty-seven observations on 10 dogs are recorded. The trend line shown in figure 1 was established by a statistical analysis and is found to be highly significant (*p* less than .01). The slope of this line is indicated by the formula Y = 17.16 + 1.69 X.

Further consideration of the factors in the experiment is necessary for a proper interpretation of the data. The factor of atelectasis of the right lung during the ob-

TABLE I. ARTERIAL BLOOD O<sub>2</sub> LEVELS, AND GAS UPTAKE AND FLOW IN BRONCHIAL ARTERY IN LUNG  
WITH LIGATED PULMONARY ARTERY

DOG NO.	SURF. AREA	MONTRS POST-OP.	Phase II			Phase III		Phase IV		MINIMAL FLOW cc/M <sup>2</sup> /min.	ESTIMATED FLOW cc/M <sup>2</sup> /min.
			GAS UPTAKE	O <sub>2</sub> AB- SORBED	N <sub>2</sub> TRANS- FERRED	BLOOD O <sub>2</sub>	BLOOD O <sub>2</sub>	GAS UPTAKE			
9	.57	6.0							18.4		
	.57	7.8							10.9		
	.57	8.0							17.6		
	.57	8.0							10.9		
	.57	9.0							6.7		
	.57	9.5							4.3		
	.59	12.0							21.0		
	.60	14.5	11.2			14.9	17.5			718	
	.61	15.0	14.1			14.5	16.4	40.0		1216	
	.61	15.5	16.2			14.5	18.2	31.9		716	
22	.62	18.0	22.3	29.7	7.4	15.7	19.2	21.2		1042	1389
	.63	20.8	10.7	17.1	6.4	17.3	19.1	43.9		911	1498
	.54	2.3							14.6		
	.54	3.3							19.3		
	.60	12.3	0.5			17.4	10.8	13.4		660	
	.60	12.5	10.2			17.5	22.2	18.3		362	
	.56	13.3	23.1			10.9	20.0	20.3		454	
	.55	14.0							11.4		
33	.56	15.0	10.1			15.7	18.5	13.8		645	
	.56	16.3	13.0	20.1	7.1	12.6	16.7	7.7		555	854
	.56	17.3	7.2	13.9	6.7	15.2	18.2	13.4		418	816
	.62	0.5							18.0		
	.62	2.0							21.0		
	.65	9.0	23.4			15.3	20.0			638	
	.66	9.1	20.6			14.3	17.1	41.0		944	
36	.67	10.0	15.1			17.1	20.5	30.1		663	
	.66	11.0	18.2			16.8	19.4	29.6		1060	
	.69	12.5	25.6	32.0	6.4	15.6	20.1	93.5		832	1032
	.60	8.0	7.8			14.4	19.1			280	
	.60	8.3	8.3			11.8	15.9	11.8		337	
37	.62	10.0	8.4	20.7	21.3	16.1	20.0			347	1230
	.69	13.5	7.9	12.4	4.5	16.6	19.2	24.0		439	683
	.75	1.0							10.5		
	.86	8.0	8.7			17.9	20.0	40.7		481	
	.84	8.3	9.9			17.6	20.9			349	
	.84	8.5	17.8			16.9	19.9	26.8		607	
	.84	9.1	14.1			17.4	19.9	63.1		671	
	.76	10.0	16.4			17.9	20.7	30.4		771	
	.76	11.1	18.0	29.8	11.8	17.3	19.6	24.8		1012	1676
	.76	13.3	17.6	33.7	16.1	16.4	19.3	13.0		818	1566

TABLE I—Continued

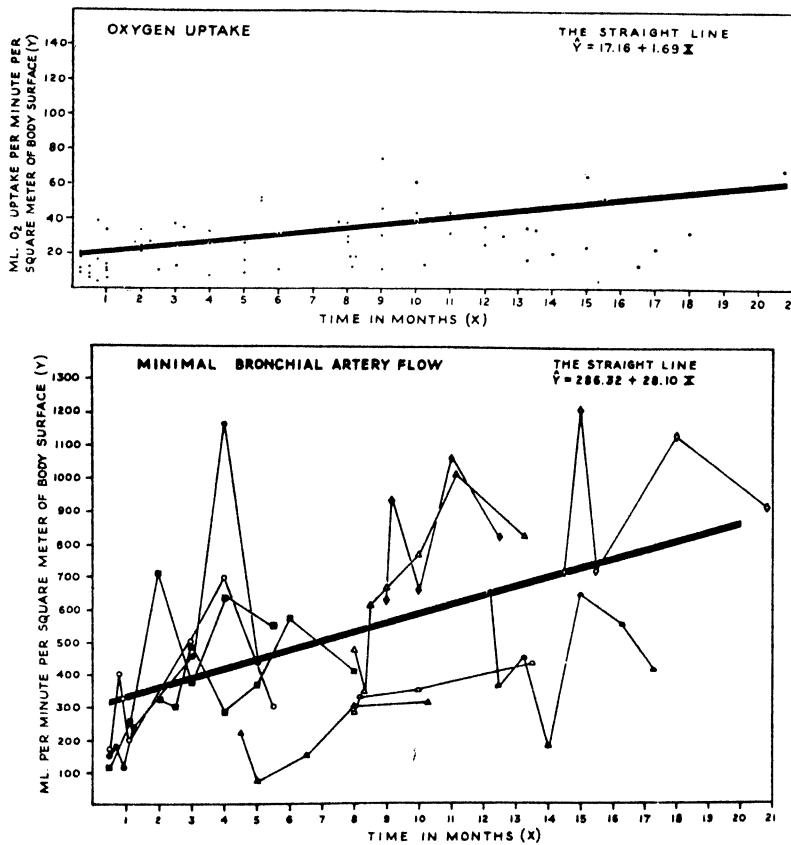
DOG NO.	SURF. AREA	MONTHS POST-OP.	Phase II			Phase III		Phase IV		MINIMAL FLOW cc/M <sub>3</sub> /min.	ESTIMATED FLOW cc/M <sub>3</sub> /min.
			GAS UPTAKE	O <sub>2</sub> ABSORBED	N <sub>2</sub> TRANSFERRED	BLOOD O <sub>2</sub>	BLOOD O <sub>2</sub>	GAS UPTAKE			
50	.72	0.8							3.1		
	.81	4.5	6.6			13.3	16.6			223	
	.74	5.0	2.4			14.4	17.4	6.9		73	
	.96	6.5	12.5			9.4	17.8			154	
	.93	8.1	6.9	13.4	6.5	13.4	15.9			301	582
	.97	10.3	10.2	15.9	5.7	14.3	17.7	14.2		313	485
52	.64	0.3							6.1		
	.64	0.5							5.1		
	.62	0.8							10.2		
	.64	2.0	3.6			14.2	15.9	13.8		331	
	.62	2.5	2.6			14.5	15.9	6.5		300	
	.64	3.0	3.9			16.4	17.6	15.8		503	
	.74	4.0	4.3			17.8	19.8	24.8		289	
	.72	5.0	5.8			17.5	19.7	11.8		367	
	.74	6.0	6.8	16.1	9.3	16.7	18.3	8.5		574	
	.76	8.0	7.3	15.0	7.7	16.6	18.9	29.3		414	845
55	.62	0.5	1.8			15.1	16.9	4.0		161	
	.61	0.8	2.4			15.9	18.1			179	
	.62	1.0	2.9			14.3	18.4	7.3		115	
	.62	1.0	4.7			14.8	18.0	3.7		237	
	.63	3.0	14.2			13.6	18.5	34.6		460	
	.63	4.0	9.2	17.5	8.3	18.5	19.8			1171	2233
	.61	5.0	5.1	16.4	11.3	17.8	19.8	16.2		430	1370
56	.62	0.5	8.9			13.1	21.4			173	
	.62	0.8	8.7			17.9	21.3	27.6		413	
	.63	1.0	12.7			14.2	20.3			332	
	.64	1.1	5.5			14.1	18.4	21.9		197	
	.75	3.0	14.7			16.4	20.4	28.4		491	
	.75	4.0	12.5	22.0	9.5	17.0	19.4	25.6		695	
	.77	5.5	6.2	11.9	5.7	20.0	22.7	39.0		299	740
57	.58	0.5	1.4			15.6	17.6	7.1		119	
	.59	1.1	5.3			13.1	16.6	6.9		256	
	.61	2.0	2.6			17.1	17.7	15.0		710	
	.59	3.0	4.9			14.9	17.1	7.7		378	
	.59	4.0	9.9	20.6	10.7	14.7	17.3	15.5		634	
	.61	5.5	6.8	12.8	6.0	16.0	18.0	31.7		544	1317
											1023

Phase II: Right lung breathes room air, left lung O<sub>2</sub> from spirometer. Phase III: Both lungs breathe O<sub>2</sub> from spirometers. Phase IV: Right lung cut off, left lung breathes O<sub>2</sub> from spirometer.

Blood O<sub>2</sub> (in femoral artery) given in vol.%. Gas uptake given in cc./min.

In phase II gas uptake based on 20-minute test, except experiment 172 (based on 30 min.) and experiment 142 (based on 7 min.). In phase III, gas uptake based on 5- or 6-minute run.

struction of its airway has already been mentioned as being in part responsible for the rising slope of the oxygen absorption line during *phase IV* (fig. 4B). When the airway of the right lung is reestablished this lung tends to reexpand, but its reexpansion may not at once reach pre-occlusive levels. Thus the estimates of oxygen absorption may be somewhat too high. The slope of the trend line, however, should not be disturbed by the factor of incomplete reexpansion of the right lung since this should be roughly the same from one determination to another.



Figs. 5 (upper) and 6 (lower)

A study of table 1 reveals that the oxygen absorption per minute during *phase II* may in some instances exceed that of *phase IV*. This suggests that there may be a fall in cardiac output which offsets the effect of the steeper oxygen gradient.

*Calculation of the Effective Bronchial Arterial Blood Flow.* By 'effective' flow is meant that flow taking part in the absorption of oxygen, i.e., the blood that reaches the capillaries of efficiently ventilated alveoli. Bruner and Schmidt (17) present evidence that in the dog, only about two-thirds of the blood in the 'bronchial

arteries' actually enters the lung. Following ligation of the pulmonary artery there is added to the blood arriving via the main bronchial arteries blood from collateral channels. As demonstrated in anatomical studies (6), this includes branches from the enlarged esophageal and pericardiophrenic vessels. For convenience in discussion, blood distributed to the alveoli from these channels is also included under 'Effective B.A.F.'

The Effective B.A.F. can be calculated from the following application of Fick's principle: Effective B.A.F. =  $\frac{a}{c - b} \times 100$ , where  $a$  is the  $O_2$  absorbed in ml/min. in the left lung,  $c$  is the  $O_2$  content of blood leaving the alveoli of the left lung, and  $b$  the  $O_2$  content of the blood in the bronchial artery in volumes per cent. If it is assumed that  $b$  is the same as the  $O_2$  content of the blood in the femoral artery from which samples can conveniently be obtained, then the only undetermined element that remains is  $c$ . This may be assumed to be the same as the  $O_2$  content of the systemic arterial blood when both lungs are breathing  $O_2$ , a value obtained by analysis in the immediately subsequent *phase III* of the experiment as has been described.

*Minimal Effective Flow.* The minimal effective flow can be calculated from data obtained during *phase II* of the experiment. By 'minimal flow' is meant that the effective blood flow is at least of the stated volume. During *phase II* the measured rise in the curve (fall in spirometer volume) represents the excess of oxygen absorbed over nitrogen transferred into the spirometer, as has been stated. Under the conditions of the experiment, the volume of oxygen absorbed always exceeds that of the nitrogen delivered. This results from the fact that at any particular blood circulation rate, the absorption gradient for oxygen, which is determined largely by the unsaturated hemoglobin entering the capillaries of the alveoli, is greater than the secretion gradient for nitrogen which results from the difference in tensions of the nitrogen dissolved in the blood and that in the spirometer. As blood flow increases this disproportion is further displaced in favor of oxygen absorption.

If the value 'a' for the formula is obtained from *phase II*, it therefore represents the minimal value for the rate of oxygen absorption. Thus a 'minimal effective bronchial arterial flow' can be calculated. The data obtained from 60 observations on 10 dogs are plotted against time in figure 6. The successive observations on a single animal are connected by lines. Statistics obtained by summing results over all 10 dogs were used in the calculation of a best-fitting linear equation representing the regression of blood-flow on time. Analysis showed that linear regression accounted for a very significant percentage of the total variation ( $p < .01$ ).

*Effective Bronchial Arterial Flow.* To obtain an estimate of the true 'effective bronchial arterial flow' the oxygen actually absorbed was determined by analysis of the gases in the spirometer at the end of *phase II*. The flow was calculated from the formula given above. Nineteen observations were made on 10 dogs. These are summarized in table 1. In 16 of the observations the flow exceeded 700 cc/ $M^2/min.$ , in 12 it was in excess of 11/ $M^2/min.$ , and in 4 it was greater than 1500 cc/ $M^2/min.$  The highest estimated flow was 2233 cc/ $M^2/min.$

#### DISCUSSION

The magnitude of the flows observed is surprising, especially in view of Bruner and Schmidt's (17) studies of normal dogs with the bubble flow meter. They found that the flows did not exceed approximately 30 cc. per minute even under maximal vasodilation induced by drugs. The large variations in flow observed in the present determinations probably depend in part on the depth of the sodium pentobarbital anesthesia. It is possible also, in some experiments, that, despite precautions, the tip of the bronchspirometric cannula may have been beyond the orifice of the left upper lobe bronchus. In Bruner and Schmidt's experiments there was evidence of a striking rhythmic vasomotor activity in the bronchial vessels of the relatively normal lung. Whether this may persist under the conditions of the present experiment is unknown. The mechanism of the remarkable expansion of the bronchial circulation that takes place after ligation of the pulmonary artery is not clear. Most of the increase in flow occurs within the first few weeks. Possibly the responsible factor is the fall in pressure within the capillary beds held in common by the two circulations when the flow in the pulmonary artery stops. Miller (12) has demonstrated these capillary beds to lie within the walls of the respiratory bronchioles. The direction of flow in these elongated fine meshed capillaries may vary depending on the relative pressures in the terminals of the bronchial and pulmonary arteries (13). Under the present conditions blood from the bronchial vessels tends to perfuse the capillaries of the alveoli where transfer of gases is efficiently accomplished.

Anatomical studies demonstrate also the opening up of easily visible pre-capillary anastomoses between the bronchial and pulmonary arteries. The reasons for their development are not clear.

Once the bronchial arteries have increased in size, by whatever mechanism, it is not difficult to understand why they should carry a large flow: the presumably low peripheral resistance in the lungs together with a high pressure at the source. Huge flows through enlarged bronchial arterial channels have also been observed in cases of congenital pulmonic stenosis (9). It is obvious, moreover, despite the absence of exact data, that the hydrostatic pressure in the capillaries of the lung supplied only by an expanded bronchial arterial circulation does not exceed the colloid osmotic pressure of the blood—else the alveoli would filter like the glomeruli of the kidney. A part of the impact of systole in the bronchial arteries is cushioned by their large communications peripherally with the persistently patent sac-like branches of the pulmonary artery.

#### SUMMARY

In the dog, a lung with a ligated pulmonary artery can maintain a respiratory function. The capacity of such a lung to absorb oxygen gradually increases over a period of months. When oxygen rather than air is supplied through a bronchospirometric cannula, while the intact lung continues to breathe air, the oxygen content of the arterial blood is found to rise. The effective flow in the bronchial arteries of such a lung increases with time after ligation, as measured by bronchospirometry and an application of the Fick principle. The increase in circulation is in step with the expansion of the vascular bed demonstrated in anatomical studies by the vinylite

method as reported elsewhere. After the fourth month with the animal under sodium pentobarbital anesthesia, the flow usually exceeds 1 l/M<sup>2</sup>/min. This flow is largely a burden on the left heart whose output becomes roughly one-third greater than that of the right. A similar situation obtains in human disease, such as bronchiectasis or congenital pulmonic stenosis, where there is an extensive collateral circulation to the lungs.

The writers wish to acknowledge their indebtedness to the late Dr. J. H. Watkins, and to Mr. E. K. Harris of the Department of Public Health, for the statistical analyses.

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## MYENTERIC REFLEX

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THE question of an enteric nervous mechanism capable of independent reflex activity has been under investigation since Nothnagel (1) and Luederitz (2) first noted that stimulation of the gut usually produces a contraction orad but not caudad. Luederitz also observed that this response could still be obtained after denervation and, therefore, considered it to be due to a local reflex which later was called the myenteric reflex by Cannon (3).

Although confirmed by Bayliss and Starling (4), partly also by Magnus (5) and Cannon (3, 6), recent investigators have cast doubt on the significance and even the existence of this reflex. In extensive studies on the rabbit, Alvarez (7, 8) could not find it. Ganter (9) and Rodin (10) and others failed to observe it in human subjects (cf. 7). Raiford and Mulinos (11, 12), on the other hand, demonstrated a reflex similar to the myenteric reflex in transplanted segments of the colon. They found that stroking the mucosa produced a contraction of the circular muscles above and of the longitudinal muscles below the stimulated region. Hukuhara (13) noted that pinching caused a stronger muscular response orad than caudad.

This question was reinvestigated using a variety of methods and preparations. The results obtained confirm the existence of an enteric reflex which involves a synaptic mechanism. It can be elicited by mild mechanical stimulation such as stroking the mucosa, or longitudinal stretching. These stimuli produced, on the oral side of the stimulated region, an augmentation of rhythmic activity or a prolonged powerful contraction with temporary cessation of rhythmic movements. This response does not progress along the intestine.

As will be shown below, the reflex response varies considerably under different conditions. The only constant characteristic of the reflex is its polarity. Therefore, in the following, all responses which show this property will be called myenteric reflex. The question whether the reflex also involves a descending inhibition, as claimed by some authors, will be discussed later.

In view of the simplicity of the experiments described below it may seem surprising that so much difficulty has been encountered in demonstrating the essential facts concerning enteric reflexes. The variability in the responses obtained is partly due to the use of abnormal, crude stimuli by many investigators. Confusion has also been introduced by the term peristalsis. The myenteric reflex comes into play in the transport of a bolus. It is erroneous, however, to assume that every wave of contraction in smooth muscle is caused by the reflex. Simple waves of contraction, such as those of the stomach and ureter, are due to muscular conduction, whereas peristalsis caused by a bolus is a much more complex phenomenon (14). It is unfortunate that the term peristalsis has been used to designate these two vastly different types of activity. The term reflex peristalsis, therefore, has been proposed for the peristaltic waves due to the myenteric reflex.

#### METHODS

Anesthetized dogs (morphine, 3 mg/kg. subcutaneously and sodium barbital, 200 mg/kg. intraperitoneally), and rabbits (sodium pento-barbital intravenously) were used. Short lengths of intestine were exposed and covered with dry cotton except during observation. The room was kept at a temperature of 27° or higher and was often humidified. Electric stimuli were applied through platinum hand electrodes. Repetitive stimuli at a frequency of 20 to 50 shocks per second, supplied by a thyrotron stimulator discharging over a transformer, were used.

Action potentials were recorded by the technique described previously (22). Monophasic potentials were obtained by placing one lead on a small mass of tissue firmly tied off by a silk thread, the other lead on another region at the same level (16). The resulting injury diminished spontaneous motility in this region but did not prevent the passage of a peristaltic wave.

#### RESULTS

*Normal Stimulus.* In the dog's intestine, lateral distension, produced by spreading a hemostat introduced into the lumen, increases the strength of the rhythmic contractions on the oral side without any noticeable effect caudally. Usually, however, a stronger response is elicited by stroking the mucosa with a blunt instrument introduced through a small opening. Stroking the serous surface on the other hand, is ineffective. It may be concluded, therefore, that specific receptors for the myenteric reflex responding to mild mechanical stimulation are present in the mucosa.

The response to stroking probably is important for the initiation of reflex peristalsis by the introduction of a bolus. That distension is not necessary is indicated by the fact that a bolus so small that it does not distend the wall of the relaxed intestine can elicit peristalsis. Furthermore, stroking the mucosa in the region of a bolus often starts a peristaltic wave under conditions where the responsiveness of the intestine is low.

Curiously enough, in the rabbit's small intestine, stroking the mucosa never elicits a response. Lateral distension is effective only in rather sensitive preparations. However, longitudinal distension, gently performed by stretching a short piece of gut with the fingers, produces strong ascending contractions of the circular muscles without any effect on the aboral side (fig. 1). In the upper part of the small intestine, the response may extend orad for 5 to 10 centimeters and may last for half a minute, whereas farther down in the intestine often only a brief contraction or none at all is produced by the stimulus. The contractions are often rhythmic, differing from spontaneous activity only by the participation of the circular muscles, or they are single contractions lasting for as long as 20 seconds.

The differences in the character of the effective stimulus in the preparations studied are probably related to the different consistency of the intestinal contents under normal conditions and may be considered an adaptation to the prevailing type of mechanical stimulus.

That smooth muscle can also be stimulated directly by stretching has been shown by a study of action potentials in the ureter (17), but, since the response so elicited

has no polarity and consists of a single all-or-none conducted impulse, it evidently has no relation to the myenteric reflex.

*Effect of Electric Stimulation.* Luederitz (2) reported that electric stimulation produced chiefly an ascending contraction in the rabbit's intestine, but Alvarez *et al.*

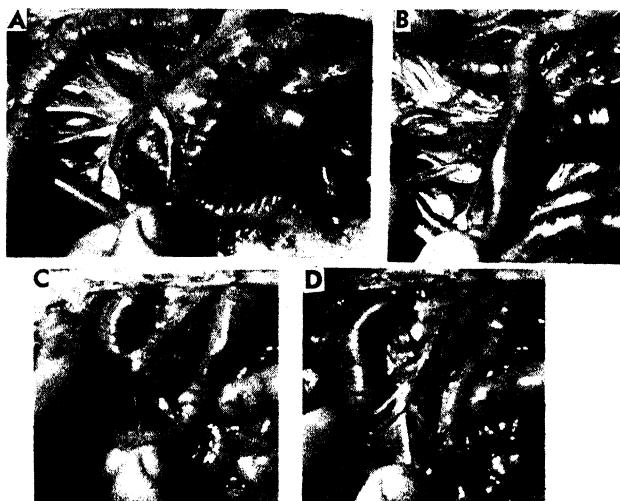


Fig. 1

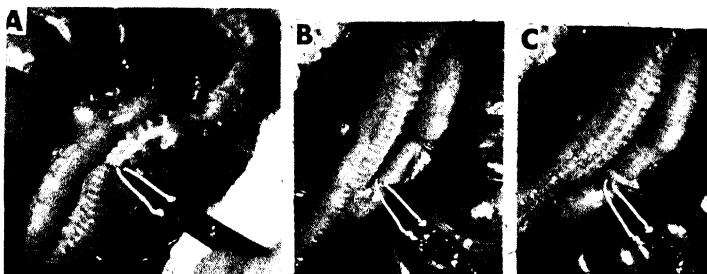


Fig. 2

(7, 18) found the responses very variable, spreading, on the average of many observations, slightly farther caudad than orad.

In my own studies, the effects of electric stimulation varied in different parts of the intestine and under different conditions, but were reproducible. Strict polarity of the response was always observed in the proximal colon of the rabbit. Electric stimulation of a small region in this part of the intestine increased the activity of the haustra, increased the tonus of the circular muscles and often caused rhythmic contractions of the longitudinal muscles orally, never on the other side (fig. 2.1).

In most parts of the rabbit's small intestine, electric stimulation can produce ascending and descending contractions. However, stimuli not more than 50 per cent above threshold always give strictly polar responses in all parts of the intestine (fig. 2B). Such weak stimuli are effective only after they have been applied for 5 to 10 seconds, showing the importance of summation in this reflex.

Weak electric and mechanical stimulation produces strong ascending and descending contractions during the period of hyperexcitability following the cessation of circulation. Similar nonpolar responses which are produced by stretching and are abolished by low concentrations of nicotine were obtained in isolated intestine by Fleisch (19).

Whether the descending response to electric stimulation, which normally has a higher threshold than the ascending contraction, is a part of the myenteric reflex

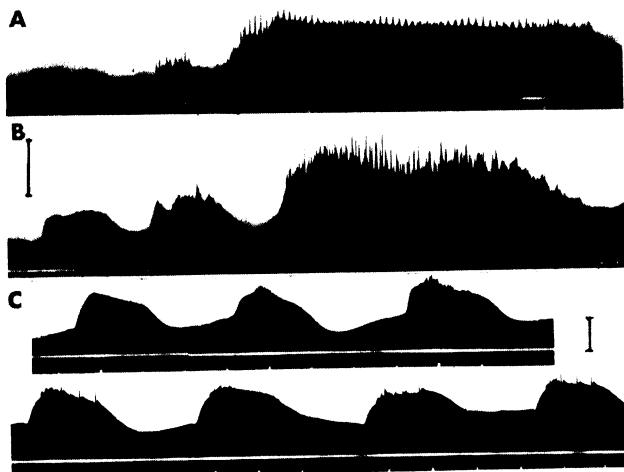


Fig. 3

belongs to some other reflex mechanism cannot be decided at present. It is possible that the polarity of the reflex is only relative, stimulation causing a quantitatively different effect orally and aborally. These considerations are important for the question of anti-peristalsis which will be discussed later.

*Muscular Response.* The character of the response varies in different preparations and depends on the strength of stimulation. In the dog's small intestine, stroking always increases the strength of the spontaneous contractions without changing their frequency. This agrees with the observations on peristalsis which, likewise, usually is rhythmic (14).

Action potentials show a characteristic difference between duodenum and ileum. In the former, spikes are very prominent and have a high frequency, whereas, in the latter, the slow potentials (20-22) are very large. In the ileum of the rabbit the reflex responses always remain rhythmic, whereas in the duodenum the response to stretching may be sustained for 8 to 10 seconds (fig. 3).

Bayliss and Starling (4) and others (3, 13) observed that pinching the intestine usually produces a descending inhibition and that a wave of inhibition precedes peristaltic waves. Auer and Krueger (23) presented evidence for a wave of inhibition preceding both peristalsis and anti-peristalsis in the colon of the rabbit. They based their conclusion mainly on the observation that, if two peristaltic waves approach each other, one or the other stops. This, however, is not a constant phenomenon, because Alvarez (7) and I have observed strong and opposing contractions on both sides of a bolus. In my experiments, the colon usually was at complete rest, except for the region orad to the bolus, as indicated by the absence of action potentials. In the dog's small intestine, electric activity and motility, as far as it could be observed visually, were not diminished ahead of a peristaltic wave.

One may conclude from the literature on the subject that descending inhibition sometimes is associated with the myenteric reflex but that it is not a constant phenomenon. It is doubtful, therefore, whether it should be considered as a part of the myenteric reflex.

The observations by Youmans, Meek and Herrin (24) perhaps have an important bearing on this problem. These authors found that distension can cause inhibition of the denervated intestine extending beyond the stimulated region. Although this effect does not seem to have any polarity, it might, under appropriate conditions, mask a descending excitatory effect or cause inhibition without suppressing the strong ascending contractions. Bayliss and Starling themselves report that pinching sometimes caused ascending as well as descending inhibition which agrees with the conclusion that inhibition is not polar and is not an essential part of the myenteric reflex.

*Coordination of Circular and Longitudinal Muscles.* In reflex peristalsis the contractions on the caudal side of a bolus usually involve only the longitudinal muscles as indicated by the fact that they do not narrow the lumen, whereas the ascending contractions constrict the intestine and cause elongation, showing that the contraction of the circular muscles is predominating. In agreement with these observations Raiford and Mulinos (11, 12) found that stimulation of the colon of the dog caused contraction of the longitudinal muscles caudad, of the circular muscles orad. The studies of Auer and Krueger (23) on the colon of the rabbit also are essentially in agreement with these conclusions.

To explain the observations just described it is not necessary to assume a separate nervous control of the two muscle layers. Quite generally, during weak contractions, chiefly the longitudinal muscles are active, producing a shortening of the intestine, whereas strong contractions produce elongation, evidently due to the greater strength of the circular muscles. The relation between the two muscle layers, therefore, may be explained on the assumption that the circular muscles have a higher threshold than the longitudinal muscles. It is possible, also, that the nervous elements responsible for the myenteric reflex make connection preferentially with the circular muscles.

*Mechanism of Conduction.* The character of the response suggests that a nervous mechanism conducting chiefly in an oral direction is involved. That muscular conduction cannot account for the effect of stimulation is confirmed by the obser-

vation that the contractions often originate at some distance from the region stimulated. In the terminal ileum of the rabbit, strong contractions often first appear 2 to 3 mm. orad to the stimulating electrodes and only later spread to the electrodes. In the duodenum, the contractions produced by longitudinal stretching often start 5 to 10 cm. above the stimulated region (fig. 1B).

The principal question is the explanation of the polarity of the response. Raiford and Mulinos have proposed that nerve fibers are running from the mucosa orad directly to the muscle fibers, thus representing an axon reflex arc. Since the effect of local stimulation extends for several centimeters, these nerve fibers must be assumed to be very long. If they had terminations on muscle fibers over their entire length, electric stimulation would be expected to produce a response orad as well as caudad. As shown above, however, the responses generally spread caudad less than a millimeter. Therefore, on the assumption of an axon reflex, it must be assumed, furthermore, that each fiber has motor endings only at one certain distance from the sensory endings.

The polarity can be explained more readily by assuming a synaptic mechanism conducting preferentially in an oral direction. This possibility can be tested by the action of drugs. It is well-known that peristalsis of the intestine, in contrast to ordinary rhythmic activity, is abolished by the intravenous injection of small quantities of nicotine, an observation which has been considered as evidence for a synaptic mechanism (4, 25). However, the effect of the drug cannot be interpreted with certainty in such experiments because the possible effects of the drug on receptors, synapses and the muscle has not been evaluated.

In the present work, drugs were applied by placing narrow strips of cotton soaked in the solutions all around the intestine. Rabbits were used. Nicotine sulfate or pure nicotine in high concentrations, 1:1000 to 1:5000, produced strong ascending and descending contractions of the circular muscles. Following this response, which passed off in one to two minutes, the response to local stimulation did not spread beyond the nicotinized region (fig. 1, 2). In concentrations of 1:10,000 to 1:20,000, nicotine usually blocked the spread of the response without first producing a contraction. All of these effects were completely reversible.

Atropine in concentrations as low as 1:10,000 and tetraethylammonium chloride as low as 1:1,500 blocked conduction like nicotine. It was unexpected that adrenalin (1:50,000 or higher) also blocked conduction in many animals. However, the minimal effective concentration of these drugs, while constant in one animal, varied considerably in different animals. In the least sensitive rabbits, 14 of a group of 26 animals, adrenalin and tetraethylammonium chloride did not block completely at concentrations which were so high (1:10,000 and 1:500 resp.) that other effects of the drugs (vascular effects and local contractions) became prominent. This group was also less sensitive to the action of nicotine and atropine.

Acetylcholine at the lowest effective concentrations produced a weak local contraction, probably due to direct stimulation of the muscle. At slightly higher concentrations (about 1:2000 for the duodenum, 1:10,000 for the lower ileum) only an ascending contraction occurred. Still higher concentrations also gave a descending

response. These observations indicate that acetylcholine stimulates the muscle directly and indirectly through a nervous plexus.

Since none of the drugs mentioned, even in concentrations much higher than those used here, stimulate or block nerve fibers, it may be concluded that the reflex responses involve a synaptic mechanism. Furthermore, because the block is sharply limited and because generally the response to stimulation does not spread caudad noticeably, the neurons involved in the reflex must be assumed to be short.

*Summation and Fatigue.* The myenteric reflex has some of the characteristics of reflexes involving the central nervous system. Brief stimulation generally is ineffective. The weaker the stimuli the longer they have to be applied before a response appears. The importance of summation is strikingly illustrated by the responses to weak electric stimuli described above.

The reflex, furthermore, is subject to rapid and long-lasting fatigue. On continuous stimulation, the reflex generally subsides within 1 minute, often within 10 seconds. Following a strong response, stimulation is entirely ineffective for half a minute or longer.

The importance of summation and fatigue is also evident from observations on peristalsis (14). The introduction of a bolus into the small intestine of the dog usually does not initiate a peristaltic wave immediately. It first produces a gradual increase of rhythmic activity on the oral side. A peristaltic wave begins only when the contractions are strong enough to propel the bolus.

#### COMMENTS

The observations described here show that the myenteric reflex involves an enteric nervous plexus which conducts decrementally, chiefly in an oral direction. It consists of short neurons connected by synapses. Pharmacologically the synapses are similar to those of the autonomic nervous system, being blocked by nicotine and tetraethylammonium chloride and stimulated by acetylcholine. The blocking action of atropine and adrenalin is peculiar but Marrazzi (26, 27) has demonstrated a depressing action of these drugs on sympathetic ganglia.

The relationship of the plexus responsible for the myenteric reflex to the autonomic system is uncertain. It appears improbable, however, that the plexus consists merely of post-ganglionic parasympathetic neurons because vagal stimulation never sets off as powerful a response as can be elicited by appropriate stimulation of the intestine.

The polar character of the myenteric reflex explains why the peristaltic waves of the intestine generally travel only in an aboral direction. The reflex by itself, however, does not set up peristaltic waves as shown by the fact that the reflex response to local stimulation never progresses along the intestine and that such waves cannot be produced in the empty intestine. Their propagation is due to the continuous stimulation by the contents of the organ which reinforces the muscular activity on the oral side, particularly that of the circular muscles.

This type of peristalsis should not be confused with the peristaltic waves of the stomach and ureter. The waves of contraction of these organs are analogous to single impulses in cardiac muscle. They can be elicited by single electric shocks.

They are all-or-none responses and are conducted equally well in both directions. These and other observations on the effect of electric currents and on action potentials (15, 20, 21, 22, 28) agree with the assumption that conduction is purely muscular. The fact that, in contrast with true peristaltic waves, conduction is not blocked by high concentrations of cocaine and nicotine (28, 29) leads to the same conclusion.

Because the action potentials of the rhythmic contractions of the intestine are essentially like those of the ureter and the stomach, it is evident that the individual contractions in all of these organs involve muscular conduction. The chief difference lies in the fact that in the intestine single contractions are generally conducted only for short distances, a condition which is largely due to the independent initiation of activity in many parts of the organ. The peristaltic waves of the intestine can travel long distances but, as shown above, they are composed of a series of rhythmic contractions, each one travelling only a short distance.

Some investigators have occasionally observed anti-peristalsis. It can be seen frequently in the rectum of the rabbit (23). In my own studies it was often seen in this organ, but only when the intestine was depressed, for instance after several peristaltic waves were induced in succession. Peristalsis, then, was weak. A wave often came to a standstill and a new wave started in the opposite direction. This phenomenon indicates that the polarity of the enteric nervous system is not absolute. There is, in fact, some evidence indicating that impulses are conducted in this system also in a caudal direction and that the difference in the response to stimulation on both sides is purely quantitative. It is conceivable, therefore, that under certain conditions, for instance in fatigue, the effect on the caudal side predominates, thereby producing anti-peristalsis.

Polarity has not been demonstrated in other visceral smooth muscles. It is true that in the ureter and stomach the waves of contraction normally travel in a caudal direction. For the ureter, probably also for the stomach, this observation is explained by the fact that the upper end of the organ has the highest degree of automaticity, thereby acting as the pacemaker.

#### SUMMARY

Local stimulation of the intestine produces responses which spread considerably beyond the stimulated area. Under certain conditions the contraction is restricted to the oral side. Such polar responses, which are designated as the myenteric reflex, may consist merely of an increase in the strength of the rhythmic contractions or they may be more prolonged contractions, chiefly of the circular muscles. The most effective stimulus for the myenteric reflex is longitudinal stretching in the small intestine of the rabbit, stroking the mucosa in the small intestine of the dog. Abnormal stimuli such as electric stimuli or pinching often produce ascending and descending contractions. However, electric stimulation always produces a strictly polar response in the proximal colon of the rabbit, and in other parts of the intestine if the stimuli are close to threshold.

Nicotine in low concentrations applied locally prevents the spread of the response from the stimulated area. Local application of acetylcholine in minimal concentrations produces a polar response like that caused by appropriate mechanical or elec-

trical stimulation. It is concluded that a synaptic mechanism consisting of short neurons and conducting chiefly in an oral direction is involved in the myenteric reflex.

The results are in agreement with the conclusion that the spontaneous contractions of the intestine are myogenic but are ordinarily conducted only for a short distance. The myenteric reflex increases this activity on the oral side and thereby propels the contents of the intestine. The reflex response by itself is not propagated. The peristaltic waves depend on the continuous stimulation by the contents of the intestine.

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# REFLEX PERISTALSIS OF THE INTESTINE

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**A**SOLID object introduced into the intestine induces powerful contractions of the circular muscles which transport the object in an aboral direction. This response has been known for a long time (1-5). In the present paper it will be described in greater detail on the basis of visual observations and records of action potentials. In a subsequent paper the underlying neuromuscular mechanism will be analyzed.

## MATERIAL AND METHODS

The small intestine of the dog and the descending colon of the rabbit were used. Dogs were anesthetized with morphine (3 mg/kg. subcutaneously) and sodium barbital (200 mg/kg. intraperitoneally). The use of morphine in the dog is important because under the influence of barbiturates alone motility is weak and peristaltic waves can be elicited only after long delays. Also without morphine, any manipulation of the intestine, the introduction of the bolus itself, produces a further diminution of motility, a reflex which is not disturbing if morphine is given. Neither purgation nor denervation, as recommended by Bayliss and Starling, was necessary. Rabbits were anesthetized with sodium pentobarbital, injected intravenously.

A piece of intestine about 6 cm. long was attached to a cork board by two pins, and longitudinal incisions were made at both ends. The board was rigidly clamped. For recording action potentials, the differential, non-polarizable electrodes previously described (8) were used in most experiments. Because some difficulty was experienced in preventing slipping during strong contractions, other types of electrodes were tried, but all gave essentially the same type of records. For very strong contractions it proved best to sew fine silk threads on the intestinal wall and connect them to the non-polarizable electrodes. Metallic electrodes directly in contact with the tissue were unsuitable because they produced large movement artifacts. For recording, an oscillograph or a mechanical recorder driven by a d.c. amplifier was used.

Varnished pieces of lead of different sizes were used as boluses. They were spindle-shaped, somewhat flattened on two sides so as to minimize the vertical movements of the intestinal wall during peristalsis. The experiments were carried out at a room temperature of 27° or higher. The air was often humidified. The exposed part of the intestine was covered with dry cotton except during observation.

## RESULTS

According to previous descriptions a peristaltic wave is simply a strong contraction traveling in an aboral direction. It was observed, however, that the response to

the introduction of a bolus is often rhythmic and that it varies in different species and even in different parts of the intestine in the same species. I shall first describe peristalsis in the small intestine of the dog.

After a bolus large enough to fill the lumen is introduced, the strength of the rhythmic contractions gradually increases on the oral side. Sometimes there is also some increase caudally, but always much less. The contractions usually originate around the posterior half of the bolus and travel from there orally. The activity on both sides of the bolus may be independent, but, as noted also by Hukuhara (3), waves of contraction originating on the oral side often travel over the bolus and beyond at a velocity of several centimeters per second. On the aboral side, the contractions are much weaker and do not involve the circular muscles. When the contractions on the oral side become strong enough to blanch the intestine, the bolus is pushed forward, each contraction transporting it for a short distance. The speed with which the bolus travels varies widely and increases, within limits, with the size of the bolus.

The contractions causing the transport of the bolus have the same frequency as those of the empty intestine and differ from the latter only by their greater strength. The peristalsis, therefore, does not represent a single wave of contractions, but rather an advancing front of strong rhythmic activity. The term 'peristaltic contraction,' commonly used for this form of activity, is not appropriate.

In very active preparations, particularly in the ileum, a more powerful response than that just described is often obtained. The bolus may travel at a speed as high as one centimeter per second, driven forward by a single powerful wave of contraction which sweeps over the whole piece of intestine under observation. In this type of peristalsis, a region of the intestine remains contracted for 10 to 20 seconds, and a piece of intestine 5 to 10 centimeters long is blanched at one time. There are, however, all intermediate stages between these waves of contraction and the rhythmic type of peristalsis described above. It seems unlikely, therefore, that any essential difference between these responses exists. Thus, the prolonged contractions may be considered as being due to a fusion of several contractions, a conclusion which is confirmed by the observations on action potentials described below.

The peristalsis of the descending colon of the rabbit has been studied by Langley and Magnus (7) and, in greater detail, by Auer and Krueger (1). This part of the intestine, in contrast to the small intestine, is completely relaxed for long periods, but spontaneous strong contractions of variable duration may occur. A bolus, if it is large enough to distend the intestine, readily induces peristalsis. This response may consist of a series of brief contractions, but more often a single prolonged contraction drives the bolus ahead. Observations by Auer and Krueger which have some bearing on the question of the neuromuscular mechanism of peristalsis will be discussed in a subsequent paper (8).

*Action Potentials.* It has been shown (6, 8) that the potentials accompanying ordinary rhythmic activity of the small intestine usually consist of a slow component, giving rise to R and T waves, and of brief spikes. The significance of this duality is not known but it was found that the strength of contraction is closely related to the magnitude and frequency of the spikes and has little relation to the size of the slow component. There are also marked and consistent differences between species and even between different regions of the intestine in the same species.

Thus, in the dog, the slow component is much more prominent in the ileum than in the duodenum (fig. 1).

The approach of a peristaltic wave has no effect on the action potentials. No evidence for a wave of inhibition preceding the peristaltic wave, as described by the older investigators (2, 7), was found. As the bolus passes below the leads the spike discharge generally increases and reaches its peak immediately oral to the bolus.

In the duodenum of the dog the action potentials at the height of peristalsis differ from those of the empty intestine only by the strong and prolonged spike discharge (fig. 1A). Although this activity may appear like a single prolonged contraction, the spikes are always grouped into distinct bursts showing that it does not differ fundamentally from the ordinary rhythmic activity of the intestine, in agreement with the conclusion already reached on the basis of other observations.

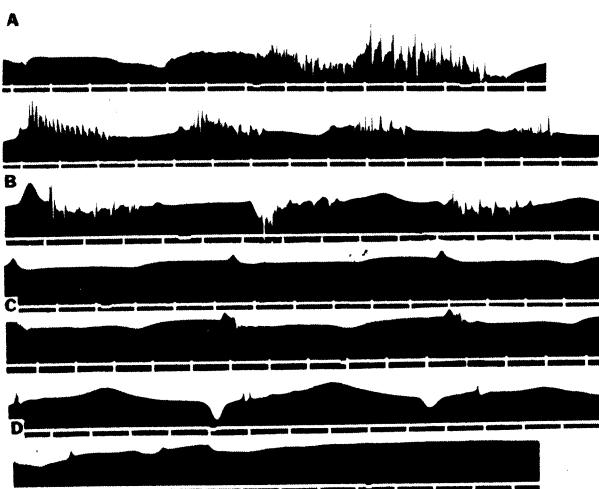


Fig. 1

In the terminal ileum, on the other hand, the spike discharge does not increase markedly during peristalsis (fig. 1B, C, D). Most surprising of all is the finding that no regular potential changes are present during the strongest contractions which cause intense blanching and last from 10 to 20 seconds (fig. 1D). Because this phenomenon was observed in dozens of cases using different types of leads, including threads sewn on the muscle, and because it never occurred in the duodenum, it seems unlikely that failure of the recording mechanism could be responsible for this unexpected result. Also various gradations from the complete absence of regular potentials to slow, but regular R and T waves, and complexes with spikes were observed. The absence of potential changes during sustained contraction shows that the muscle can remain in a continuous state of activity for rather long periods of time so that no external potential differences are present, a situation similar to that during the iso-electric phase of the electrocardiogram and of the action potentials of many visceral smooth muscles.

During the spontaneous contractions of the rectum of the rabbit, brief bursts of spikes, which seem to be superimposed on slower rhythmic potential changes, are discharged at regular intervals (fig. 2A). The frequency of the bursts is about one per second but increases with the strength of contraction. During the periods of rest, no potential changes can be detected, but small slow potential waves of the same frequency as the bursts often precede a discharge. The peristaltic waves are accompanied by the same type of action potentials as those of the empty organ but the frequency of the bursts may be as high as two per second at the beginning of the contraction, declining gradually later on (fig. 2B, C).

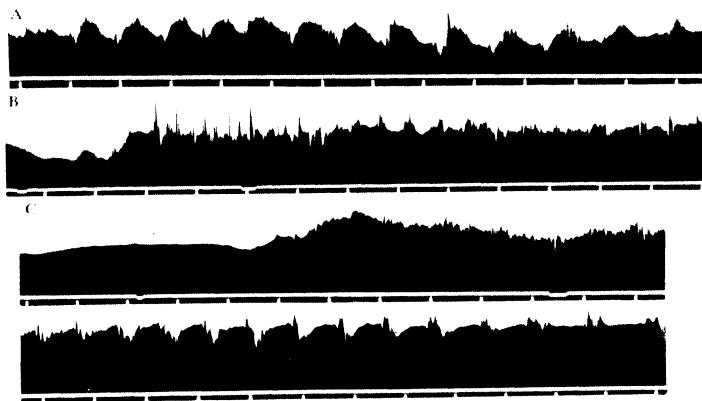


Fig. 2

#### COMMENTS

Since peristalsis usually is a rhythmic form of activity, it is evident that it is not comparable to the peristaltic contractions of the stomach and ureter. The latter are all or none conducted and correspond to the individual contractions which make up the peristaltic waves of the intestine. This difference is confirmed by observations on the action of drugs which will be discussed in a subsequent paper (8). The results obtained suggest that the peristaltic waves of the intestine involve a synaptic reflex mechanism whereas there is no evidence for any participation of nervous elements in the individual contractions of the ureter and stomach. The first of these types of activity, to distinguish it from simple waves of contraction, may appropriately be called reflex peristalsis because it involves a nervous mechanism.

The differences in the responses of the intestine in different species and in different parts of the digestive tract are remarkable but they do not necessarily indicate any fundamental differences in the underlying mechanisms. They also emphasize the importance of carrying out investigations on more than one type of preparation.

#### SUMMARY

In the duodenum of the dog the introduction of a bolus strongly increases the strength of the rhythmic contractions on the oral side, with little or no effect caudally

This activity differs from spontaneous contractions only by their greater strength and participation of the circular muscles. In strong peristalsis a single powerful and prolonged contraction sweeps over the intestine. The latter type of response is seen frequently in the rectum of the rabbit and terminal ileum of the dog, less often in the duodenum of the dog. Since all gradations between these responses can be observed, the prolonged contractions may be considered as the result of a fusion between several rhythmic contractions. This view is confirmed by a study of action potentials which differ usually from ordinary rhythmic contractions only by the greater strength of the spike discharge. However, in the ileum of the dog, no regular action potentials were observed during the strongest peristaltic waves, indicating a prolonged and continuous state of activity.

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# THE AMERICAN JOURNAL OF PHYSIOLOGY

*Published by*  
THE AMERICAN PHYSIOLOGICAL SOCIETY

VOLUME 157

June 1949

NUMBER 3

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## MEASUREMENTS OF HEART OUTPUT BY ELECTROKYMOGRAPHY<sup>1</sup>

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ASIMPLE method for accurately measuring heart output is greatly needed but up to the present time, no completely acceptable procedure has been found. The ballistocardiograph is simple and requires very little cooperation from the subject. Many, however, do not consider it reliable (1, 2, 3). Roentgenkymography (4-7) is another simple method but the subjective errors in measuring the x-ray plates may be very large—perhaps as much as 12 per cent. Electrokymography avoids the subjective errors of measurement since its records are sharp. Furthermore, the amplitude of the movements of the heart borders may be greatly amplified. One cannot decide at the present time whether this method will prove to be as good as or better than ballistocardiography or roentgenkymography. In developing this procedure, we have compared it with ballistocardiography.

### METHOD

The top of a fluoroscopic table has been removed from its base and supported by 4 vertical springs. This provides a ballistic table similar to the one described by Nickerson, Warren and Brennan (8). Movements of the table top are recorded by means of a light beam reflected from a pivoted mirror onto bromide paper. Using the same camera, the movements of the string galvanometer activated by the electrokymograph (9) are simultaneously recorded. The electrokymograph head is provided with an opening 3 cm. in diameter through which x-rays can pass to excite a fluorescent screen. A wafer grid, attached beneath this screen, eliminates the effects

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Received for publication February 18, 1949.

<sup>1</sup> The funds for this research were provided by R. G. 194-C2, U. S. Public Health Service.

of scattered x-rays. Enclosed in the same housing and 4 cm. above the wafer grid is the 931-A photomultiplier tube (fig. 1). Beneath the top of the table, a solenoid is arranged to swing presswood (6 mm. thick) into and out of the x-ray beam. This is used to calibrate the movements of the heart recorded by the electrokymograph (see fig. 2). For measuring the thickness of the heart, a phantom of presswood 5 cm. thick is provided.

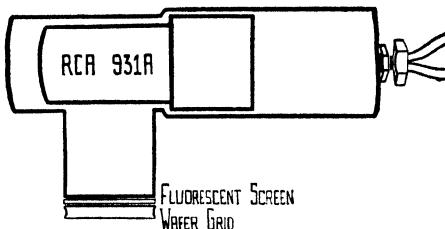


FIG. 1. ELECTROKYMOGRAPH HEAD.

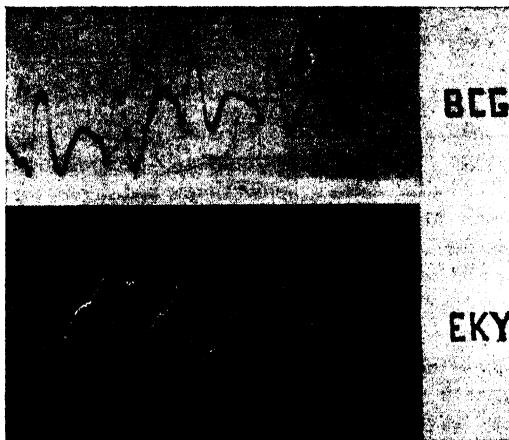


FIG. 2

The principles involved in the method used are best described by an example. When water is placed between the x-ray tube and the electrokymograph head, the amount of x-ray transmittance is indicated by the response of the string galvanometer resulting from moving the calibrator into the x-ray beam. The greater the depth of water the less the transmittance of x-rays and the smaller the calibrating response. As would be expected, a graph of these responses on semi-logarithmic paper gives a straight line (fig. 3). Thus, by means of the responses produced by the calibrator, one can measure any changes in the thickness of the water. Since muscle and blood absorb nearly the same amount of x-rays as water, it follows that one should be able to measure the dorso-ventral thickness of the heart by the method described above. First one calibrates with the electrokymographic (EKY) head over the ventricular

shadow. The head is then moved to the right of the spine shadow over chest walls and lungs only and the calibrations are repeated. In most cases, the transmittance of x-rays is so large when the heart is not in the field that the phantom for the heart (see above) must be placed between the x-ray tube and the subject. This absorbs x-rays to about the same extent as the heart. To determine the thickness of the heart, the points along the line on figure 3 which correspond to the calibrating responses over the heart on the left and over the chest and phantom on the right are found and the thickness of water to which these correspond is noted. Then, since the presswood of the phantom absorbs x-rays to the same extent as water or blood, its thickness (5 cm.) is added to that determined from figure 3. This gives the systolic thickness of the heart.

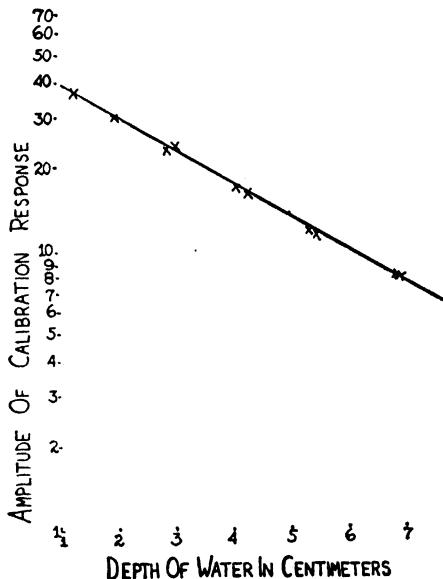


FIG. 3

When the EKY head is over the ventricles, it records the antero-posterior movements of the ventricular walls. If the calibrations are timed so as to occur at the end of systole, then they cause the string to move toward the position it takes during diastole. When the antero-posterior movements are small, a calibration will move the string beyond its diastolic position and with large ventricular movements, the calibration will not quite return the string to the diastolic level. In figure 2, the calibrations are shown for every other beat. The amplitude of the cardiac record is 1.06 times that of a calibration. Since a calibration corresponds to the x-ray absorption of 0.625 cm. of water, the change in AP thickness of the heart amounts to 0.66 cm. For small differences a linear relation between the calibrating response and the change in AP thickness may be assumed. For larger differences, it should be remembered that this is a logarithmic function.

The procedure followed in these experiments is as follows: The subject lies on the table with his feet firmly against the foot board. He is then told to take a deep breath and with fluoroscopic guidance, the EKY head is placed over the heart shadow and slightly nearer the apex than the base. Care is taken not to have any

TABLE I

	DIASTOLIC THICKNESS	SYSTOLIC THICKNESS	STROKE CALCULATED FROM		% DEVIATION <sup>1</sup>
			EKY	BCG	
	cm.	cm.			
<b>WOMEN</b>					
SHE	5.98	5.35	88	84	+ 5
SPI	5.95	5.31	88	64	+ 27
WUR	6.27	5.52	107	105	+ 1
RYA	7.88	7.09	145	139	+ 4
TRU	5.48	4.93	70	85	- 17
	5.42	4.90	66	82	- 19
WEB	6.26	5.69	83	81	+ 2
VIT	5.07	5.00	87	102	- 15
MIL	5.59	5.05	70	74	- 5
CAN	6.11	5.82	43	91	- 53
	5.73	5.33	54	94	- 42
PEA	6.27	5.63	93	89	+ 4
<b>MEN</b>					
FLA	7.78	7.22	102	110	- 8
MUS	6.57	6.04	82	116	- 29
KEL	7.94	7.43	95	95	0
FRI	8.07	7.49	110	109	+ 1
McA	7.08	6.56	87	85	+ 2
DON	7.77	7.00	139	132	+ 5
DEL	7.49	6.86	110	112	- 2
DEA	6.52	5.89	95	86	+ 9
CUT	6.56	5.74	123	132	- 7
RAB	6.91	6.21	111	106	+ 5
POW	6.71	6.06	101	122	- 17
	6.41	5.64	113	122	- 7
POL	8.52	7.92	121	116	+ 4
MYE	6.54	6.05	75	84	- 11
LON	8.00	7.47	100	103	- 3
RIC	6.01	5.17	115	111	+ 4
	6.22	5.35	123	118	+ 5
CHA	6.43	5.66	113	100	+ 13
EVA, B.R.	7.73	7.15	106	86	+ 22
FIE	7.54	6.99	93	74	+ 32

<sup>1</sup> Difference between EKY and BCG results.

part of the head over the spine shadow. The amplification in the galvanometer circuit is adjusted to give a suitable record. After this the subject is allowed to breathe until the rate returns to normal. The subject is then instructed to take a deep breath and hold it while the ballistocardiograms and EKY densograms are simultaneously

recorded. The experimenter constantly watches the shadow of the galvanometer string and puts in a calibration at the end of every other systole. He records 2 strips of about 30 beats each during breath holding. Then the head is moved about 5 mm. away from what was judged to be the ideal position for recording. The purpose of this is to see whether the densograms recorded in the second position differ from those obtained first. A series of responses to calibrations over the chest walls and lungs to the right of spine is next recorded as indicated above. From this result and the calibrations over the heart the antero-posterior thickness of the heart is determined.

The x-ray tube was operated at 80 kv. and  $2\frac{1}{2}$  ma. This produced 8 r per minute at the patient's skin. The exposure in the area under the heart averaged 3 minutes and on the right side of the chest  $1\frac{1}{2}$  minutes. Since the x-rays were coned down as soon as the head was properly placed, the total exposure of any skin area was very

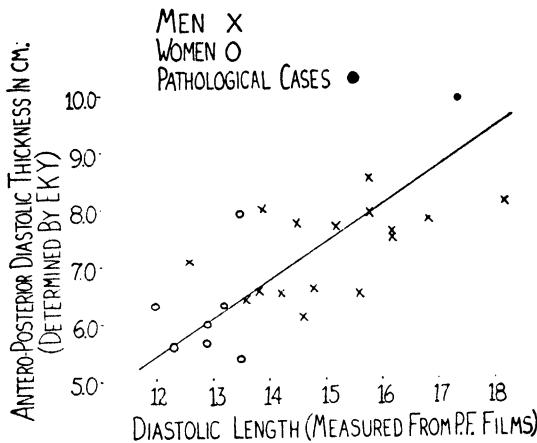


FIG. 4

small; therefore, frequent repetition of the procedure is possible without appreciable harm to the patient.

#### RESULTS

In table 1 is shown the thickness of the heart of each subject (medical students and nurses) in systole and diastole. These figures are a measure of the average thickness of that portion of the heart under the electrokymograph head. The measurements made from lateral roentgenograms would be larger than these, for then one is measuring from the right side of the heart which gives the ventral contour to the left side which makes up the dorsal contour. The thickness measured at any point on the anterior surface to a point directly posterior would be less than the figure obtained from such roentgenograms (10).

That our results are consistent with those determined from measurements of x-ray shadows is shown by figure 4. For this graph the heart shadow on a photofluorographic (PF) film has been enlarged to full size and the long diameter measured. One would expect considerable scatter in the results since these films were taken

sometime before the electrokymograph records and are not entirely satisfactory for measuring heart size. The results, nevertheless, do show a consistent trend.

To calculate the stroke volume (S.V.) from observations using the EKY, we have developed a formula which would give the best fit with the ballistocardiographic results. This formula is  $S.V. = 12.2 (T^2 \text{ diast.} - T^2 \text{ syst.})$  where  $T$  is the antero-posterior thickness as measured with the electrokymograph. If the heart were a

TABLE 2. THE ANTERO-POSTERIOR DENSITY CHANGE PER CALIBRATION

	POSITION 1	POSITION 2	AV.	% DEVIATION
<b>WOMEN</b>				
CAN	.50	.51	.505	1
SHE	.98	1.03	1.005	2
WUR	1.12	1.27	1.195	7
SPI	1.04	1.03	1.035	1
TAY	.70	.60	.65	8
ROU	.86	.87	.865	1
RAM	.93	1.12	1.025	8
VIT	1.07	1.08	1.075	1
WEB	.99	.92	.955	4
<b>MEN</b>				
CAR	1.28	1.40	1.34	4
PIN	1.29	1.35	1.32	2
COO	1.06	1.02	1.04	2
TRU	.87	.84	.855	2
RUS	.84	.76	.80	5
SOU	.82	.82	.82	0
SHE	.45	.48	.465	3
STE	1.51	1.66	1.585	5
PAU	.59	.75	.67	12
MIL	.87	.86	.865	1
MUS	.94	.77	.855	10
MYE	.82	.73	.775	6
LON	.90	.78	.84	7
POW	1.04	1.24	1.14	9
RIC	1.35	1.40	1.375	2
FRI	.91	.96	.935	3
CHA	1.26	1.18	1.22	3
EVA	.97	.86	.915	6
FIE	.88	.88	.88	0
FLA	.92	.87	.895	3
DEA	.88	.93	.905	3
CUT	1.17	1.22	1.195	2

sphere in systole and diastole and if one were measuring the true diameter, then the exponent for  $T$  should be 3. Neither of the above premises is correct, and it turns out that our best fit is obtained using the exponent 2.

One questions whether the electrokymographic records are reproducible. To test this, we have moved the head away from the position considered most satisfactory. In table 2, the results are shown. The differences are not in every case due

entirely to change in position of the head. In a number of subjects, the output of the heart changed and accounted for part of the difference. Furthermore, in some subjects the calibrations were not placed so that they always included the end of systole. Thus, there were occasionally too few calibrations to give a reliable average. An automatic device to calibrate at the proper time would, we feel, reduce the differences shown in this series, which now average 4 per cent.

The position of the heart within the chest changes during each systole and one must consider whether this has an important effect on the records. When recording the movements of the lateral borders of the heart, positional changes play a large part in the results obtained. With the EKY head placed at about the middle of the ventricular shadow, the positional movements have far less effect though the rotation of the heart to the right probably means that more of both the right and left ventricles come under the EKY head. Furthermore, the base of the heart moves toward the apex during contraction. Thus, the recorded movement may well be less than the antero-posterior change in thickness of the heart at any point on its surface.

Another source of the difficulty is the chest calibrations. It is impossible to be sure that the EKY head covers the same amount of the shadow of ribs, muscle and lungs on the right and on the left. Nevertheless, this does not seem to result in any large error. In each subject we have made chest calibrations in 2 positions. One is a centimeter cephalad of the other. In our calculations of output we have averaged these calibrations. If instead one uses one of the extremes the maximal difference in output is found to be 3 per cent. In most cases it averaged less than 1 per cent.

The differences between our results and those obtained using the ballistocardiograph are small (table 1). Eighty per cent of the EKY results differ from ballistic calculations by less than 20 per cent. The ballistic method checks with the Fick (8) no better than this. Therefore, it is possible that the EKY gives results as good as or better than the ballistocardiograph. The real test of the method will come when it is checked against the Fick or the Stewart method.

#### DISCUSSION

The strokes given in table 1 were not obtained under basal conditions. The subjects were not post absorptive, had not rested for half an hour before the observations were begun, and furthermore the stroke of the heart was affected by suspension of respiration. In this preliminary work, we were interested only in comparing the output by the two methods. If our method proves satisfactory, it can be adapted to basal conditions and in many individuals will not require breath holding.

One may question whether the EKY head is over the ventricles only and is therefore recording their movements alone. If the subject were standing, the left atrium would extend so far downward that a part of it would probably lie between the x-ray tube and the EKY head. With subject supine, the apex drops far enough so that the atrium probably does not intercept the x-rays activating the EKY. Even if part of the x-ray beam is intercepted by the left atrium, the movement of the base of the heart toward the apex during ejection elongates the atria and may balance out any effect on antero-posterior thickness of these chambers which is produced by the entrance of blood (12).

We are now recording densograms with the subject in the left lateral position as well as supine. In the lateral position the apex probably moves downward enough so that here also the atria do not interfere with the ventricular recording. With measurements of movements and thickness in two dimensions, the accuracy of the methods is increased. Furthermore it is probable that we shall be able to calculate heart size from these records. If so, another means of determining the importance of Starling's Law in the human heart will be available; for evidence in dogs see (11).

A well nourished ventricle, working against a normal arterial pressure and receiving an adequate venous inflow, contracts considerably during ejection of blood. On the other hand, a ventricle which is in poor condition is able to contract very little and must dilate to maintain an adequate output. In a group of healthy individuals, if athletes with thick walled ventricles were omitted, we might also find that those whose hearts were large for the size of their bodies, had ventricles which contracted

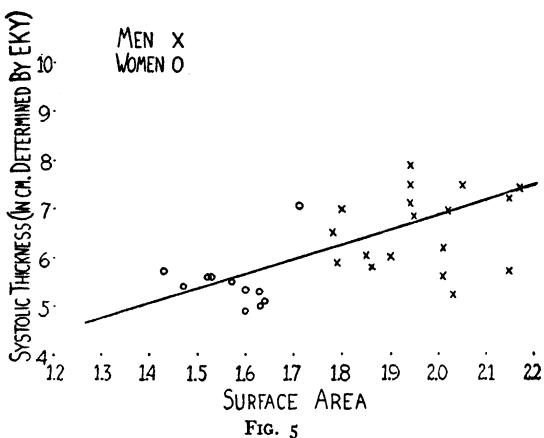


FIG. 5

little during ejection of blood. To test this, the systolic thickness of the heart of each of our subjects was plotted against his surface area (fig. 5). A line was then drawn on this graph which showed the average thickness of the heart for persons of any size. From this graph and the AP thickness of each person's heart, the per cent of predicted heart size was calculated. Those whose hearts were 80 to 90 per cent of the predicted size showed an average change in thickness during systole of 11.2 per cent; hearts 90 to 100 per cent of normal size showed a change of 9.3 per cent; those 100 to 110 per cent of normal showed an 8.6 per cent change and those 110 to 120 per cent of normal showed an 8.0 change. Thus, the size of a heart and its ability to contract are related. The results suggest that change in thickness of the heart with ejection may be a measure of cardiac condition.

#### CONCLUSIONS

A method for measuring the output of the heart using the EKY has been described.

This method gives a measure of the AP thickness of the heart both in systole and diastole (see table 1).

An inverse relation between thickness of the heart and its contractile power is suggested from these observations.

We wish to express our thanks to Doctor George C. Henny and Doctor Robert R. Newell of the Department of Radiology, Stanford University Hospital, San Francisco, Calif., who offered suggestions during the carrying out of these experiments.

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# AN EVALUATION OF THE CARDIOVASCULAR EFFECTS OF CERTAIN DRUGS IN HYPOTENSIVE DOGS<sup>1</sup>

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**N**UMEROUS attempts have been made to find drugs that would be beneficial in the treatment of hypotensive states and 'shock' (1-4). The majority of studies have been confined to the effects of various pressor agents on arterial and venous pressures or on survival time. As Frank *et al.* (1) have stated, "it has been assumed that with an increased arterial pressure resulting from vasoconstriction there is a redistribution of blood through the critical circuits." Measuring the effects on arterial pressure caused by a particular drug gives no information on whether or not blood is being distributed to critical circuits, since variations in arterial pressure are reflections of the summation of changes in the peripheral vascular bed (vasoconstriction or vasodilatation) and cardiac output. For example, if a given drug produces an increase in arterial pressure and an increase in cardiac output, with a decreased total peripheral resistance (peripheral vasodilatation), the efficiency of such a drug in effecting satisfactory circulation to vital organs of the body can be assumed to be higher than one which produces an increase in blood pressure due to an increase in total peripheral resistance (peripheral vasoconstriction) alone.

With these concepts in mind the effects of Aranthol (2-methylamino-6-hydroxy-6 methyl heptane 2-methylamino iso-octanol), Oenethyl (2-methylamino heptane), epinephrine, amphetamine and theophylline on cardiac output, total peripheral resistance (T.P.R.), as well as mean blood pressure were examined in hypotensive dogs.

## METHODS

Dogs anesthetized with 300 mg./kg. of sodium barbital were bled rapidly from a femoral artery until the mean blood pressure was approximately 50 mm. Hg, as determined by a mercury manometer in the other femoral artery. They were maintained at this level for about 90 minutes with additional bleeding or infusion of blood as needed. Usually the blood pressure stabilized within 15 to 30 minutes after the initial bleeding. The drug to be tested was given intravenously at the end of the 90-minute period and cardiac outputs determined for a varying length of time, usually 120 minutes. At the end of the experiment the dogs were transfused to determine whether or not irreversible shock had been precipitated. Dogs found to be in the irreversible stage are not included in this report.

Cardiac outputs were determined from pressure pulses obtained from the right carotid artery by the method of Hamilton and Remington (5). This method has

Received for publication February 28, 1949.

<sup>1</sup> This investigation was aided by a grant from Bilhuber-Knoll Corporation. The theophylline was supplied by The G. D. Searle Company.

been found to give satisfactory results when compared to the direct Fick method (6). Total peripheral resistance was calculated by the formula (7)

$$\text{TPR} = \frac{\text{Pm} \times 1332}{\text{C.O./sec.}} = \frac{\text{dynes sec.}}{\text{cm}^5}$$

The figures given for cardiac output are expressed in percentages of values at the end of the hypotensive period or values immediately before a drug was administered.

### RESULTS

The effect of Aranthol was observed in 12 hypotensive dogs. A single dose of 10 mg/kg. consistently produced a prompt and prolonged increase in cardiac output (fig. 1). The average increase in cardiac output was about 20 per cent and the duration of action from 85 to 240 minutes. Changes in T.P.R., mean pressure, and heart rate are illustrated in figure 1. In the hypotensive dog 20 mg/kg. appears to produce a maximal increase in cardiac output. Further doses then fail to produce any detectable change until the output begins to fall. When this occurs Aranthol will again augment the output.

TABLE I. EFFECTS ON CARDIAC OUTPUT

DRUG	AVERAGE % CHANGE FROM END OF HEMORRHAGE	AVERAGE DURATION OF ACTION, MIN.
Aranthol.....	+21	120
Oenethyl.....	+15	60
Epinephrine.....	+20	3
Amphetamine.....	+10	120
Theophylline.....	-35	60

Oenethyl (10 dogs) in a dosage range of 2.5 to 10 mg/kg. increases the cardiac output, but the effect is more irregular and less prolonged than with Aranthol. The mean pressure change is variable and the T.P.R. decreases. Successive doses of Oenethyl evoke decreasing responses from the cardiovascular system.

The administration of amphetamine (7 dogs) in doses of 1 to 5 mg/kg. produced an average increase in cardiac output of 10 per cent for the 120-minute period. A small but consistent fall in mean pressure was observed, and the T.P.R. fell below the hypotensive level after an initial steep rise (fig. 2).

Theophylline (8 dogs), 5 mg/kg., characteristically produced a considerable decrease in cardiac output, a drop in mean pressure and a small increase in T.P.R.

A prompt and marked rise in cardiac output, T.P.R., mean pressure and heart rate occurs when epinephrine, 0.05 mg/kg., is administered. The duration of these effects is brief, usually less than 5 minutes.

### DISCUSSION

The cardiac output in hypotensive dogs was increased by Aranthol, Oenethyl, epinephrine, and amphetamine. The comparative effects of these drugs on cardiac output are presented in table I. As one would anticipate, the duration of action of

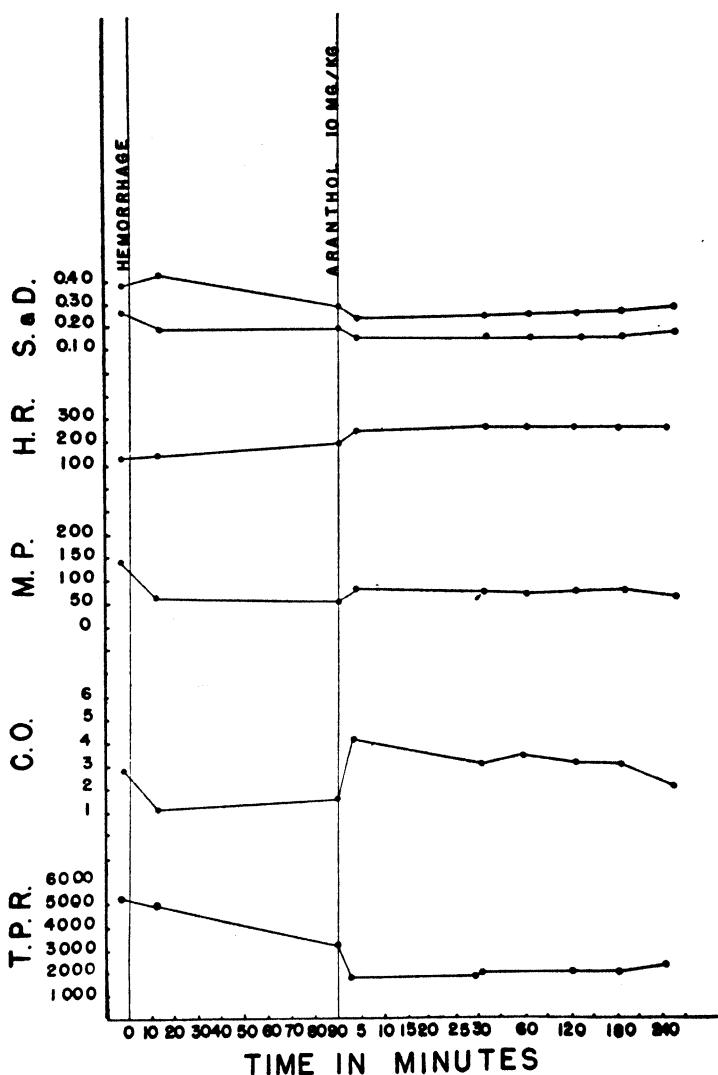


Fig. 1. THE FIRST SET OF POINTS gives the control values. Hemorrhage occurred at 0 time. T.P.R., total peripheral resistance in absolute units; C.O., cardiac output in liters/min./M<sup>2</sup> body surface; M.P., mean pressure in mm. Hg; H.R., heart rate; S. & D., systolic and diastolic duration.

epinephrine is transitory. Theophylline produced a decrease in cardiac output of such magnitude as to result in a fall in mean pressure despite an increase (slight) in T.P.R.

Amphetamine, after a latent period of about 5 minutes, increased cardiac output appreciably. However, there is always a preliminary decrease below the hypotensive

level, and further, the mean pressure for 15 to 30 minutes after the injection of amphetamine falls to a very dangerous level. It is of interest to note that the effect

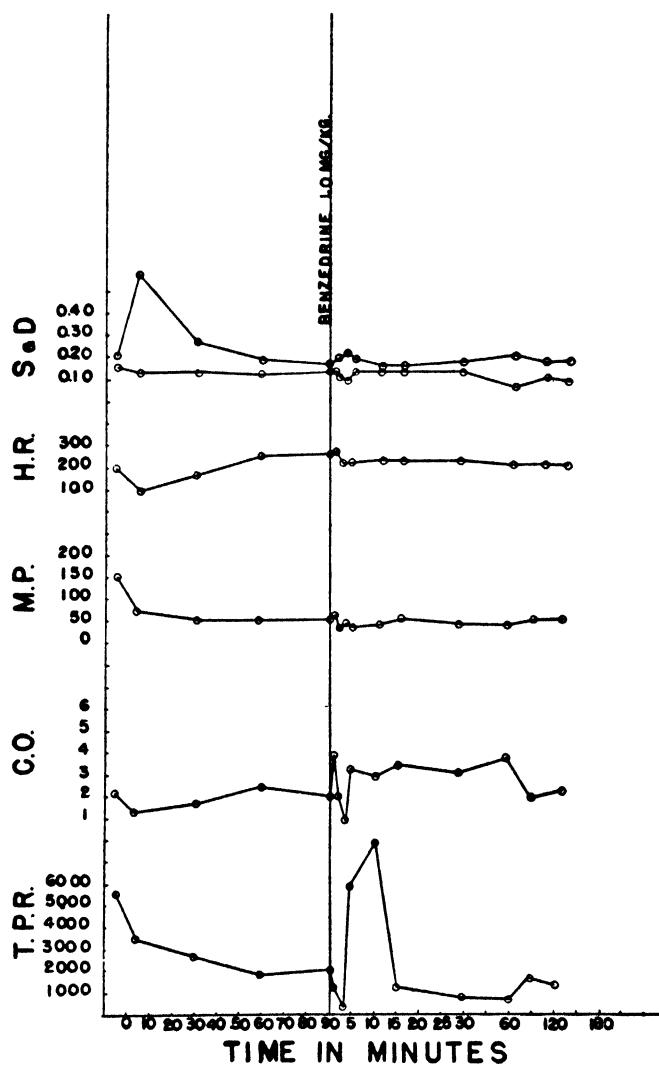


Fig. 2. SYMBOLS the same as in fig. 1

of amphetamine in the hypotensive dog is the direct opposite of that expected from results on normal dogs.

Oenethyl produces a significant and prolonged increase in cardiac output, but the increase is more erratic than that produced by Aranthol. Also, successive doses have

a decreasing effect on output. As Jackson (8) and Ahlquist (9) have shown tachyphylaxis occurs, limiting the usefulness to the first dose.

Aranthol has been reported (10-12) to cause a marked and prolonged increase in force of contraction and heart rate with an increase in blood pressure. In the hypotensive dog there is a marked increase in cardiac output which is associated with a decrease or only slight increase in T.P.R. Therefore, the improved circulatory dynamics appear to be largely cardiac in origin, and with no peripheral vasoconstriction; the improved circulatory efficiency should be reflected throughout the vascular system. The onset of the effects of Aranthol are almost immediate and the duration of its action is prolonged. The properties suggest that Aranthol might be of value in the treatment of hypotensive conditions.

The data on the duration of systole and diastole yielded little pertinent information. In about 60 per cent of the dogs, during hemorrhage, a marked increase in the length of diastole and decrease in systolic (figs. 1 and 2) duration occurred.

#### SUMMARY

Aranthol, Oenethyl, epinephrine, amphetamine and theophylline were administered intravenously in varying doses to dogs rendered hypotensive by bleeding to 50 mm. Hg for 90 minutes. Data on the effects of these drugs on total peripheral resistance, cardiac output, mean pressure, heart rate and the duration of systole and diastole are presented. Aranthol, Oenethyl, amphetamine and epinephrine increase the cardiac output in hypotensive dogs, while theophylline consistently reduces the output. The effects of Aranthol and Oenethyl are greater and more sustained than any of the other drugs tested. Oenethyl produces a more erratic response and, in addition, tachyphylaxis occurs which is not apparent with Aranthol.

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# EXCRETION OF SOLUTES AND OSMOTIC WORK OF THE 'RESTING' KIDNEY OF HYDROPEMIC MAN

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**T**HE energy requirement for the production of urine was a subject of great interest to the early workers in renal physiology. In the absence of exact knowledge of the path of urine production, and of the intermediate processes of renal metabolism, they were led to consider first the thermodynamically necessary work required to produce the differences in solute concentration between plasma and urine (1, 2). On the basis of general principles of thermodynamics and the gas laws the formula was derived

$$W = RT V (U \ln U/P + P - U) \quad (1)$$

where  $W$  indicates the work,  $R$  is the gas constant,  $T$  the absolute temperature,  $U$  the urinary and  $P$  the plasma concentration of a solute and  $V$  is the urinary flow. (See also Newburgh, 3.) The work value thus derived refers to the idealized process of urine formation conducted in a thermodynamically reversible manner, without energy losses due to back diffusion, heat production, metabolic side processes or irreversible reactions, in short at 100 per cent efficiency. It represents the theoretical minimal work necessary to produce urine. It was pointed out by Rhorer (2), that this work consisted not alone in producing from plasma a fluid of differing total osmolarity, but that the concentrations of each individual solute had to be considered. Later, Borsook and Winegarden (4) calculated in a more exact manner than preceding workers the theoretical osmotic work of urine production. Most recently Newburgh has presented a discussion of changes which tend to reduce the renal osmotic work. Obviously there are two theoretical minima for renal work: 1) if urine and plasma are of the same composition, in which case no osmotic work (but still filtration work) need be done; and 2) if urine excretion ceases. Newburgh (3) pointed out that changes in disease, such as increases of blood urea, tended to decrease the osmotic work required to clear the body of a given quantity of solute.

As part of a general inquiry into the osmotic limitations of the kidney it appeared of interest to investigate experimentally the renal work during hydropenia and osmotic diuresis. The following questions, among others, were posed: Is there an over-all maximum of renal osmotic work during hydropenia and forced diuresis? If so, is it the same for loading with different solutes? Under what conditions of urine flow does such a maximum occur? Is there any relation between urine osmolarity and renal osmotic work performed or the work capacity? The present communica-

tion deals with the pattern of solute excretion and the renal osmotic work of hydro-  
penic man, under 'resting' conditions, i.e., without solute loading.

#### METHODS

The subjects were boys 8 to 15 years of age with normal renal function and without major disease. They were fasting and had received no water for 16 hours prior to experimentation. One or two blood samples were obtained on each subject during the one or two periods of urine collection which extended for approximately 30 minutes. Plasma and urine were analyzed for urea, sodium, potassium, chloride, phosphate, and in some instances sulfate. On urine the total osmolarity was also determined. The methods and calculations used have been described (5).

#### RESULTS AND DISCUSSION

*Solute Excretion.* In table 1 are given the mean values with their standard errors of the concentration of solutes in plasma and urine of 15 subjects, who were studied over 26 periods of urine collection in 17 experiments. Also given is the mean value for urine flow, corrected to  $1.73 \text{ m}^2$  body surface. It may be readily seen that urea accounted for about one-half and the electrolytes for the other half of the osmolarity of urine. The sum of the solutes determined accounted on the average for 84 per cent of the total osmolarity, calculated from the freezing point depression by a procedure outlined previously (5). The magnitude of individual variations of each of the urine solutes should be pointed out. The urine flow, as well as the concentration of electrolytes, ranged from values of 0.5 to 1.5 of the mean. Urea was the most constant solute, with about two thirds of the variation of the electrolytes. Some of the variation is undoubtedly due to differences in the preceding dietary intake.

The plasma values other than the total calculated osmolarity deserve little comment. Its value was calculated by the procedure outlined in the footnote to the table. A similar value is obtained if the normal value for  $\text{CO}_2$  content, 27 m.Osm/l, and for the sum of Ca and Mg, 3.5, is added to the sum of the solutes determined.

A comparison of the data for urine with those in the literature is of interest. Similar rates of flow have been observed by McCance (6) and Gamble (7), but Chesley (8) under conditions of prolonged thirsting and fasting observed smaller volumes. The total osmolarity, as well as the concentration of the individual solutes, is similar to the values measured by McCance (6) on two adult subjects, but lower than the maximum achieved during a 6-day period of water deprivation in the experiments of Gamble (7).

*Osmotic Work.* In table 2 are summarized the data on the minimal renal work of the subjects, calculated by equation 1. An inspection of the distribution of the values indicates that urea accounted for the bulk, approximately two thirds, of the work. Potassium was next in importance. Together these two solutes accounted for 90 per cent of the total work. Sodium and chloride were lowest in the list. It is of interest to contrast the distribution of the work values with that of the concentrations. By the nature of the equation it is the ratio between plasma and urine, rather than the absolute urine concentration, that determines the renal work.

To evaluate the significance of the total work value, reference should be made to the work of the kidney under other conditions. In osmotic diuresis during hydro-

TABLE I. PLASMA AND URINE CONCENTRATIONS OF SOLUTES  
*Means and standard errors of 26 periods on 15 subjects in 17 experiments.*  
*Concentrations are expressed in milliosmols per liter.*

SPECIES-MEN	URINE VOL. cc/min/ 1.73 M <sup>1</sup>	UREA				Na	K	Cl	PO <sub>4</sub>	SO <sub>4</sub>	SUM SOLUTES DETER.	TRUE TOTAL OSMOLARITY CALC.
		NH <sub>4</sub> <sup>+</sup>	Cl <sup>-</sup>	Na <sup>+</sup>	K <sup>+</sup>							
Urine	0.49±0.03	500±17	153±12	143±10	162±12	38.0±3.0	26.3±0.8	986	±23	1182±18		
Plasma		7.3±0.4	148±2.0	4.9±0.2	105±1.1	1.5±0.1	1.1±0.1	267.8±3	304 <sup>1</sup>			

<sup>1</sup>The value for total osmolarity of the plasma was estimated as  $2(\text{Na}^+ + \text{K}^+) - 8 + (\text{urea})$ . The formula is based on the simplifying assumption that the proteins are the only osmotically negligible ions and that urea is the only non-electrolyte. The total osmolarity of the electrolytes expressed in terms of cation equivalents is  $2(\text{Na}^+ + \text{K}^+) + 1.5(\text{Ca}^+ + \text{Mg}^+) - (\text{Prot})$ . Substitution of the values of 7 mEq/l. for the sum of Ca and Mg, and of 18 for protein, leads to the previous expression.

TABLE 2. IDEAL OSMOTIC WORK OF RESTING HYDROGENIC KIDNEY<sup>1</sup>  
*Means and standard errors of 26 experimental periods. Work values are expressed in cal/min/173 M<sup>2</sup>*

URINE VOL. cc/min/ 1.73 M <sup>1</sup>	UREA				Na	K	Cl	PO <sub>4</sub>	SO <sub>4</sub>	TOTAL <sup>2</sup> WORK	WORK/CC. URINE
	NH <sub>4</sub> <sup>+</sup>	Cl <sup>-</sup>	Na <sup>+</sup>	K <sup>+</sup>							
0.49±0.03	0.47±0.03	0.004±0.001	0.10±0.01	0.006±0.002	0.024±0.002	0.018±0.003	0.63±0.03	1.30			

<sup>1</sup>Work was calculated according to equation 1. Plasma values were each multiplied by 1.05 to give an assumed value for the concentration in the glomerular filtrate. For expression of the work in cal/min. the concentration of each solute in plasma and urine was expressed in osmols/l., and the urine volume, corrected to 1.73 M<sup>2</sup> body surface, in l/min. A value of 1.986 cal. was used for R, and 31° abs. for T.

<sup>2</sup>The value includes 0.008 cal./min. for the combined work for HCO<sub>3</sub> and glucose calculated on the assumption of a value for their sum of 25 m.Osm/l. in the plasma, and zero in the urine.

penia, even though the urine osmolarity decreases, the calculated work rises to 4 cal/min., about 7 times as high as the value in the present experiments. A second question is how the work in the hydropenic 'resting' state compares with that under normal, 'eu-hydrouric' conditions of urine flow. Such a comparison is presented in table 3. It contains a series of work values for different rates of urine flow, calculated under the simplifying assumption of a constant solute load and constant plasma concentration of the solutes. Such an assumption introduces an error only with respect to urea. The urea work with rising urine flows is under-estimated, since with hydration the plasma concentration of urea decreases and the clearance increases. It may be seen from the table that with flow increasing to the eu-hydrouric range, the total work value diminishes slightly to reach a minimum at 2 cc/minute. It rises again slightly with further increase in volume. The relative constancy of the total work is a consequence of compensating changes in the values for the different solutes. As the work for Na, Cl, and the sum of bicarbonate and glucose rises, that for the other solutes falls. The general pattern of work curves for constant load and variable volume has been described schematically by Newburgh (3).

TABLE 3. CALCULATED OSMOTIC WORK FOR CONSTANT SOLUTE LOAD AND PLASMA CONCENTRATIONS  
AND VARYING URINE FLOW<sup>1</sup>  
*Values are expressed in cal/min/1.73 M<sup>2</sup>*

URINE FLOW cc/min/ 1.73 M <sup>2</sup>	UREA	Na	K	Cl	PO <sub>4</sub>	SO <sub>4</sub>	GLUCOSE <sup>2</sup> AND HCO <sub>3</sub>	TOTAL
0.5	.47	.004	.10	.006	.024	.018	.008	.630
1.0	.39	.015	.075	.003	.018	.012	.015	.528
1.5	.33	.044	.059	.016	.014	.009	.023	.495
2.0	.28	.078	.048	.036	.011	.007	.031	.491
2.5	.25	.116	.037	.059	.009	.006	.038	.515

<sup>1</sup> The solute load was assumed to consist of the average values determined for the condition of hydropenia. <sup>2</sup> See footnote 2 in table 2.

One may conclude that under the conditions here described, i.e. of maximal osmolarity and minimal urine volume, the kidneys are in a relatively resting state. Their thermodynamic work exceeds only little that done under eu-hydrouric conditions.

The absence of relationship between urinary osmolarity and renal work should be emphasized. In the literature it has been frequently assumed, either expressly (6) or by implication (9), that a maximal urinary concentration is either synonymous with, or indicative of, a state of maximal renal work. Such an assumption is not only contradicted by the results of the present study but is actually theoretically unjustified. The factors defining the maximal urinary concentration or the ratio between urine and plasma osmolarities are as yet unknown. They may be a function of the thermodynamic work necessary to produce a given concentration difference, or they may be related to an over-all osmotic ceiling, possibly dependent on the permeability of the distal tubular wall. Even if the first case held, the concentration difference by itself could not be a measure of the work being done. The situation would be closely comparable to the case of a concentration cell. The con-

centration difference, i.e. the potential, by itself no measure of the energy output, has to be multiplied by flow (current) to yield a work value. Although under conditions of minimal urine flow, as under conditions of minimal electrical flow, the potential is maximal, the output is only a fraction of the possible maximum. If the second hypothesis is applied, namely that an over-all osmotic ceiling related to the structure of the distal tubular wall governs the maximum osmolarity, no conclusion at all can be drawn from the osmolarity with regard to the total osmotic work.

The question as to whether or not one can predict the maximal work value, i.e. the work capacity, from that of the resting kidney cannot be answered as yet. Off-hand there is no relationship apparent but it remains for further studies to determine whether such an association exists, and what its nature may be.

Finally the relation of the theoretical work to the actual metabolism of the kidney should be discussed. It was stressed in the introduction that the calculated work refers to a thermodynamically reversible process, conducted at 100 per cent efficiency. Actually, the thermodynamic work has been found in various experimental animals to account for only 1 to 2 per cent of the total metabolism of the kidney, as measured by its oxygen consumption (10-12). No direct measurements of renal efficiency are available in man. An approximate estimate may be given on the basis of the reported oxygen consumption of the 'resting' human kidney, determined to be 10 cc./minute by Bradley and Halperin (13). Assuming a R.Q. of 1.0, so that 1 cc. of oxygen corresponds to 5 cal., the total energy available to the human kidney would then amount to 50 cal. The average value of thermodynamic work here reported, 0.6 cal./min., corresponds to 1.2 per cent of the total energy, a value in agreement with the previously cited figures for experimental animals. A similar estimate of the efficiency of the human kidney was reached in an indirect manner by Borsook and Winegarden (4). Suffice it to say here, that there exists considerable doubt as to whether any relation obtains between the thermodynamic work and the total metabolism of the kidney.

#### SUMMARY

The solute excretion and the renal osmotic work in man in the hydropenic state has been presented. It is concluded that the kidney under conditions of minimal urine flow and maximal osmolarity is in a relatively resting state. The osmotic work is only little more than that under normal, eu-hydrouric conditions.

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# EXCRETION OF SOLUTES AND OSMOTIC WORK DURING OSMOTIC DIURESIS OF HYDROGENIC MAN. THE IDEAL AND THE PROXIMAL AND DISTAL TUBULAR WORK; THE BIOLOGICAL MAXIMUM OF WORK

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**P**REVIOUS papers dealt with the relations between urine flow and excretion of solutes in hydrogenic man during osmotic diuresis produced by 11 different loading solutes (1, 2). In this communication different aspects of these experiments will be presented. The pattern of excretion of the individual loading solutes, the relations between urine and plasma concentrations, and the electrolyte losses will be considered. Furthermore, calculation of the renal work, both of the ideal process and of the work for the proximal and distal portions of the renal tubule, is included.

## METHODS

The experimental procedures and the chemical methods used have been described previously (1, 3). The material of this paper includes 214 urine collection periods observed during 26 experiments on 21 subjects, of whom 3 were diabetic. Five of the subjects were studied twice. All loading solutes except xylose, sorbose and sorbitol were used in more than one experiment each. Glucose loading was carried out only in diabetic subjects. Mannitol was used as the loading solute in 5 experiments and creatinine in 3. In several of the experiments with NaCl, creatinine and glucose, a large dose of solute was given orally prior to its intravenous administration. In one experiment urea was given orally followed two hours later by glucose both orally and intravenously. In another experiment the order of administration of the two solutes was reversed. For calculation of the dose of the electrolytes in osmols, the molecular weight was divided by the number of ions constituting the molecule.

The calculation of the ideal renal osmotic work by the equation

$$W = RT V (U \ln U/P + P - U) \quad (1)$$

where W indicates the work, R is the gas constant, T the absolute temperature, U the urinary and P the plasma concentration of a solute and V is the urinary flow has been described previously (3, footnote 1, table 2).

## RESULTS

### *Pattern of Solute Change in Plasma and Urine during Osmotic Diuresis*

In table 1 are summarized representative experiments on 8 of the 11 solutes studied. They are: urea, creatinine, NaCl, sodium sulfate, sodium para-aminohippurate, mannitol, sucrose and glucose. Omitted are loading experiments with

Received for publication January 17, 1949.

TABLE I. URINE FLOW AND SOLUTE CONCENTRATIONS IN PLASMA AND URINE DURING OSMOTIC DIURESIS PRODUCED BY VARIOUS LOADING SOLUTES  
*All concentrations are expressed as m. Osm/l.*

C. B., 11 yrs., 28.9 kg., 1.08 M<sup>2</sup>

## UREA

PERIOD NO. AND SPECIMEN	CONCURRENT TIME, MIN.	URINE VOL., CC/MIN/ 1.73 M <sup>2</sup>	UREA	Na	K	Cl	PO <sub>4</sub>	SO <sub>4</sub>	SUM SOLUTES DETER.	TOTAL OSMO- LARITY CALC.*
P-2 U	-47 to -5	0.50	552.1	225.0	106.5	172.2	34.8	23.3	1114	1179
P	-14		8.4	145.5	5.8	103.6			263	303

*o to 15 I. V. injection 52% urea, 1385 m.Osm/1.73 M<sup>2</sup> in 46 cc. saline*

2 U	38 to 59	4.58	556.7	50.0	15.8	37.0	4.1	2.3	666	686
P <sup>1</sup>	29		53.7	149.2	8.8	106.4		1.2	319	362
P <sup>2</sup>	48		50.1	146.2	6.5	102.8		1.1	307	348
3 U	59 to 78	4.61	561.0	42.5	15.0	33.0	5.4	2.6	660	700
P	68		47.0	144.7	5.0	106.4		0.9	304	338

G. P., 13 yrs., 42.5 kg., 1.34 M<sup>2</sup>

## CREATININE

PERIOD NO. AND SPECIMEN	CONCURRENT TIME, MIN.	URINE VOL., CC/MIN/ 1.73 M <sup>2</sup>	CREATI- NINE	UREA	Na	K	Cl	PO <sub>4</sub>	SO <sub>4</sub>	SUM SOLUTES DETER.	TOTAL OSMO- LARITY CALC.*
P-1 U	-101 to -66	0.66		466.0	67.5	125.3	112.5	41.6	26.9	840	1029
P	-87			6.81	150.0	4.9	103.6	1.6		267	309

*o to 4 I.V. injection 7.6% creatinine, 144 m.Osm/1.73 M<sup>2</sup> with 50 cc. saline*

3 U	5 to 25	6.97	261.2	143.8	52.5	39.5	79.0	8.4	4.6	589	661
P	15		8.8	6.81	146.3	5.0	102.8			270	310
4 U	25 to 46	3.51	276.2	183.9	65.0	46.3	87.0	11.3	6.6	676	756
P	35		6.5	6.81	150.0	5.7	102.0			271	317

*48 to 56 I.V. injection 7.6% creatinine 218 m.Osm/1.73 M<sup>2</sup>*

5 U	58 to 79	7.96	273.2	79.0	50.0	34.0	73.0	5.5	3.0	518	632
P	68		15.3	6.81	148.5	5.4	103.2	1.7		281	322
6 U	79 to 100	4.97	315.8	120.0	45.0	39.0	72.6	8.4	4.7	606	710
P	89		11.1	6.81	145.5	5.3	102.8			272	312

W. H., 11 yrs., 31.2 kg., 1.12 M<sup>2</sup>

## NaCl

PERIOD NO. AND SPECIMEN	CONCURRENT TIME, MIN.	URINE VOL., CC/ MIN/1.73 M <sup>2</sup>	Na	Cl	UREA	K	PO <sub>4</sub>	SO <sub>4</sub>	SUM SOLUTES DETER.	TOTAL OS- MOLARITY CALC.*
1 U	71 to 100	8.36	275.0	262.0	78.9	24.5	6.1	3.5	650	684
P	85		168.8	130.8	4.8	3.5	1.3	1.2	310	341

*o to 27.5 I.V. injection 10.4% NaCl, 1052 m.Osm/1.73 M<sup>2</sup> with 30 cc. saline*

2 U	100 to 121	6.34	255.0	249.6	67.0	35.2	5.2	3.1	615	666
P	112		170.0	120.6		4.4		1.4	305	346
3 U	121 to 142	6.34	247.5	241.0	68.9	43.8	4.8	2.8	608	728
P	141.5		166.2	124.0	4.7	3.7	1.3	1.1	301	336

TABLE I—Continued

L. R., 14 yrs., 38.0 kg., 1.30 M<sup>2</sup>Na<sub>2</sub>SO<sub>4</sub>

PERIOD NO. AND SPECIMEN	CONCURRENT TIME, MIN.	URINE VOL., CC/ MIN/1.73 M <sup>2</sup>	UREA	Na	K	Cl	PO <sub>4</sub>	SO <sub>4</sub>	SUM SOLUTES DETER.	TOTAL OS-MOLARITY CALC. <sup>2</sup>
<i>o to 6 I.V. injection 18% Na<sub>2</sub>SO<sub>4</sub>, 758 m.Osm/1.73 M<sup>2</sup> with 50 cc. saline</i>										
1 U	19 to 47	8.94	61.1	355.0	41.5	8.0	2.5	106.2	664	708
P	30		7.4	167.0	6.2	100.0	1.2	12.6	294	346
2 U	47 to 66	5.31	88.8	393.0	63.0	4.6	3.6	175.7	729	779
P	57		8.1	167.0	6.2	102.4		8.6	292	346

*71 to 78 I.V. injection 18% Na<sub>2</sub>SO<sub>4</sub>, 506 m.Osm/1.73 M<sup>2</sup>*

PERIOD NO. AND SPECIMEN	CONCURRENT TIME, MIN.	URINE VOL., CC/ MIN/1.73 M <sup>2</sup>	PAH	UREA	Na	K	Cl	PO <sub>4</sub>	SUM SOLUTES DETER.	TOTAL OS-MOLARITY CALC. <sup>2</sup>
3 U	81 to 107	10.6	45.9	368.0	35.8	9.8	1.9	194.8	656	690
P	91		7.0	179.0	6.5	102.0		13.9	308	370
4 U	107 to 127	7.45	70.6	395.0	53.3	2.0	2.9	187.2	711	772
P	117		7.3	170.0	5.6		1.2	11.0	195	350

A. R., 10 yrs., 28.9 kg., 1.02 M<sup>2</sup>

Na p-aminobenzoate

PERIOD NO. AND SPECIMEN	CONCURRENT TIME, MIN.	URINE VOL., CC/ MIN/1.73 M <sup>2</sup>	PAH	UREA	Na	K	Cl	PO <sub>4</sub>	SUM SOLUTES DETER.	TOTAL OS-MOLARITY CALC. <sup>2</sup>
<i>o to 2 I.V. injection of 20% NaPAH, 113 m.Osm/1.73 M<sup>2</sup></i>										
2 U	21 to 41	2.77	240.0	110.5	248.8	31.4	29.4	23.2	683	819
P	29		2.8	7.3	151.5	5.7	104.0		271	314

*49 to 51 I.V. injection of 20% NaPAH, 220 m.Osm/1.73 M<sup>2</sup>*

PERIOD NO. AND SPECIMEN	CONCURRENT TIME, MIN.	URINE VOL., CC/ MIN/1.73 M <sup>2</sup>	PAH	UREA	Na	K	Cl	PO <sub>4</sub>	SUM SOLUTES DETER.	TOTAL OS-MOLARITY CALC. <sup>2</sup>
4 U	74 to 95	4.35	232.0	91.8	248.8	26.8	41.4	14.2	655	684
P	84		6.7	6.0	159.8	5.8	101.2	1.3	281	329
5 U	95 to 122	3.52	238.4	110.6	240.0	43.8	38.6	19.7	691	742
P	107		4.6	6.2	159.0	5.7	100.8		276	328

A. R., 10 yrs., 28.9 kg., 1.02 M<sup>2</sup>

MANNITOL

PERIOD NO. AND SPECIMEN	CONCURRENT TIME, MIN.	URINE VOL., CC/ MIN/1.73 M <sup>2</sup>	MANNI-TOL	UREA	Na	K	Cl	PO <sub>4</sub>	SUM SOLUTES DETER.	TOTAL OS-MOLARITY CALC. <sup>2</sup>
<i>o to 7 I.V. injection 25% mannitol, 467 m.Osm/1.73 M<sup>2</sup></i>										
1 U	9 to 30	10.3	331.3	43.0	32.5	12.3	51.0	2.1	472	522
P	21		28.2	7.0	141.8	4.8	95.6	1.7 <sup>1</sup>	279	320

2 U	30 to 49	7.29	360.4	67.2	37.5	12.3	58.8	4.0	540	623
P	40		23.8	7.6	141.8	4.8	98.8	1.7 <sup>1</sup>	278	317

*50 to 57 I.V. injection 25% mannitol, 467 m.Osm/1.73 M<sup>2</sup>*

PERIOD NO. AND SPECIMEN	CONCURRENT TIME, MIN.	URINE VOL., CC/ MIN/1.73 M <sup>2</sup>	MANNI-TOL	UREA	Na	K	Cl	PO <sub>4</sub>	SUM SOLUTES DETER.	TOTAL OS-MOLARITY CALC. <sup>2</sup>
3 U	58 to 80	17.7	327.2	27.0	30.0	6.0	47.6	2.1	440	486
P	71		49.2	7.0	134.3	5.0	90.8	1.7 <sup>1</sup>	288	327

4 U	80 to 99	13.7	325.3	27.5	37.5	8.8	52.8	3.2	455	539
P	90		40.2	7.6	137.3	5.6	94.0	1.7 <sup>1</sup>	286	326

*101 to 108 I.V. injection 25% mannitol, 467 m.Osm/1.73 M<sup>2</sup>*

PERIOD NO. AND SPECIMEN	CONCURRENT TIME, MIN.	URINE VOL., CC/ MIN/1.73 M <sup>2</sup>	MANNI-TOL	UREA	Na	K	Cl	PO <sub>4</sub>	SUM SOLUTES DETER.	TOTAL OS-MOLARITY CALC. <sup>2</sup>
5 U	109 to 130	22.8	313.7	22.6	30.0	8.8	46.2	1.7	423	476
P	122		58.0	8.6	131.3	4.7	92.4	1.7 <sup>1</sup>	297	331

6 U	130 to 149	14.4	352.8	30.1	27.5	12.0	45.6	2.4	470	524
P	139		48.9	7.3	132.8	4.5	90.4	1.7 <sup>1</sup>	286	323

TABLE I—Continued

D. W., 12 yrs., 26.4 kg., 1.06 M<sup>2</sup>

## SUCROSE

PERIOD NO. AND SPECIMEN	CONCURRENT TIME, MIN.	URINE VOL., CC/MIN/1.73 M <sup>2</sup>	SUCROSE	UREA	Na	K	Cl	PO <sub>4</sub>	SUM SOLUTES DETER.	TOTAL OSMOLARITY CALC. <sup>1</sup>
<i>o to 42 I.V. injection 74.4% sucrose, 392 m.Osm/1.73 M<sup>2</sup></i>										
1 U	47 to 68	14.8	284.0	28.2	46.0	4.1	46.4	1.9	411	501
P	55.2		41.3	5.6	136.3	4.1	98.8	1.3	287	320
2 U	68 to 88	10.6	308.2	48.4	43.0	7.2	47.8	2.4	457	556
P	77.8		33.2	5.5	137.5	4.2	101.6		282	314

*go to 112 I.V. injection 74.4% sucrose, 196 m.Osm/1.73 M<sup>2</sup>*

3 U	114 to 141	15.3	292.1	34.4	44.0	8.0	47.8	2.2	428	522
P	129.5		41.9	5.9	140.0	4.1	101.6		294	328
4 U	141 to 161	10.6	327.2	40.0	43.5	14.2	47.4	3.0	475	590
P	151.2		34.0	6.2	142.5	4.5	102.4	1.5	291	326

L. D., 13 yrs., 38.9 kg., 1.31 M<sup>2</sup>

## GLUCOSE

PERIOD NO. AND SPECIMEN	CONCURRENT TIME, MIN.	URINE VOL., CC/MIN/1.73 M <sup>2</sup>	GLUCOSE	UREA	Na	K	Cl	PO <sub>4</sub>	SO <sub>4</sub>	SUM SOLUTES DETER.	TOTAL OSMOLARITY CALC. <sup>1</sup>
P	-76		17.2	7.3	141.0	4.7	102.4	0.9		274	308
P-2 U	-61 to -38	0.86	468.9	275.3	180.0	54.0	127.5	7.7	14.4	1128	1149

*-24 to -23 glucose, oral, 366 m.Osm/1.73 M<sup>2</sup> in 70 cc. lemon juice**o to 4 I.V. injection 50% glucose, 256 m.Osm/1.73 M<sup>2</sup> with 50 cc. saline*

1 U	5 to 26	9.04	348.2	61.4	82.5	14.2	58.4	1.4	2.1	568	618
P	15		38.0	7.3	132.0	5.6	100.0	0.9		284	312
2 U	26 to 46	7.79	353.8	71.1	92.5	13.5	70.0	1.8	2.5	605	660
P	37		38.7	8.8	134.0	5.5	100.0			287	318

*52 to 56 I.V. injection 50% glucose, 476 m.Osm/1.73 M<sup>2</sup>*

3 U	58 to 79	18.5	313.5	31.4	82.5	15.5	59.6	1.0	1.8	505	529
P	69		55.8	8.8	127.5	5.8	98.0			296	323

<sup>1</sup> Determination on pooled plasma.

<sup>2</sup> The value for total osmolarity of the plasma was estimated as  $2(\text{Na} + \text{K}) - 8 + (\text{urea})$ . The formula is based on the assumption that the proteins are the only osmotically negligible ions and that urea is the only non-electrolyte. The total osmolarity of the electrolytes may be expressed in terms of cation equivalents as  $2(\text{Na} + \text{K}) + 1.5(\text{Ca} + \text{Mg}) - \text{Prot}$ . Substitution of the values of 7 mEq/l for the sum of calcium and Mg, and of 18 for protein, leads to the previous expression.

xylose, sorbose and sorbitol. To conserve space, only two preliminary periods on the first two experiments and one in the case of a diabetic subject have been included. Also, all late post-loading periods and those with rapidly changing plasma levels during and immediately following injection of the loading solute have been omitted. The table contains information on the age, weight and surface area of the subjects and on the size of the dose administered. The tabulated data comprise the urine

volume, the concentrations in plasma and urine of the loading solute, as well as of urea, sodium, potassium, chloride, phosphate and, in most instances, sulfate. The sum of the solutes determined and the calculated total osmolarity are also given. The value for urine was estimated from the freezing point depression in a manner previously described (2), and that for plasma was calculated in the manner indicated in the footnote to the table.

It may be seen that the urine flows following loading increased in a widely varying manner from the pre-loading rate of about 0.5 ml/min. (3). The maximum rate was 22.8 ml/min. The smallest response was observed with urea and NaPAH and the greatest with mannitol. A comparison of the amounts administered indicates that the diuretic response bore no direct relation to the dose administered. The factors entering into relation have been discussed previously (2).

The total osmolarity of urine and plasma may be considered next. The calculated plasma osmolarity increased from a mean pre-loading value of 304 m. Osm/l. to as much as 370 m. Osm/l. in the case of urea, and to 362 m. Osm/l. in the case of sodium sulfate, increases of about 20 per cent above the basal level. The mean level for all diuretic periods was about 330 m. Osm/l. The total osmolarity of the urine decreased in all experiments during diuresis in a pattern discussed extensively elsewhere (1, 2).

The concentration of the loading solutes in the plasma increased by as much as 58 m. Osm/l. in the case of the mannitol experiment presented. In another experiment with mannitol a peak value of 62 m. Osm/l. was observed. Other large increases occurred with sucrose, glucose and the other sugars and sugar alcohols, substances which distribute themselves in a small volume of body fluid. A high plasma concentration was also observed in the urea experiment, following a very large dose. The increase in sodium was about 35 m. Osm/l. in the case of sodium sulfate, and was somewhat smaller with sodium chloride loading. With para-aminohippurate and creatinine the increase in the plasma was least marked, in the former case because of the small dose administered and a rapid rate of excretion, and in the latter because of a large distribution volume. The urine concentrations of the loading solutes varied considerably. In the case of urea the concentration remained constant at the pre-loading level despite a nine-fold increase of urine flow. It is noteworthy that the maximum urine concentration of all other nonelectrolytes tended to average about 330 m. Osm/l. The sodium concentration reached a maximum of nearly 400 m. Osm/l. with sodium sulfate, a value in excess of any previously reported. In the case of loading with sodium chloride a lower maximum of sodium, approximately 275 m. Osm/l. was reached, similar to values reported by McCance *et al.* (4). Of the anions, chloride reached the highest urine concentration. In one experiment, not presented in the table, a concentration of 370 m. Osm/l. was reached at a rate of urine flow of approximately 2 cc/min. following a dose of sodium chloride of 590 m. Osm/1.73 M<sup>2</sup>, a value in exact agreement with that reported by Davies *et al.* (5) and McCance (4). In the experiment presented, a lower value was measured at a much higher rate of urine flow. Para-aminohippurate and sulfate had lower maximum concentrations. It is of interest that the concentration of all loading solutes tended to decrease somewhat at the highest urine flows.

The urinary concentrations of the solutes other than the loading solute on the

whole decreased as expected with increasing urine flow. Several significant exceptions are noteworthy. In the experiments with sodium sulfate and sodium chloride the potassium concentration decreased much less than expected. This behavior of potassium following sodium administration is well known and needs no further emphasis. With sodium sulfate loading the chloride concentration decreased to lower levels than observed in any other experiment. This effect is similar to the phenomenon observed in dogs by Lotspeich (6). He found that the administration of chloride interfered with the reabsorption of sulfate. The results of the other diuretic experiments here presented would suggest that the relation between chloride and sulfate is of a specific nature rather than based on a non-specific osmotic effect.

A more complete presentation of the excretion of the electrolytes during osmotic diuresis is summarized in the 4 graphs constituting figure 1; the amounts of sodium, chloride, potassium and phosphate, in m. Osm/min. are plotted against the rate of urine flow for all 11 loading solutes. In figure 1A the sodium excretion, excluding loading experiments with sodium salts, is presented. Despite a great variability of the data, a clear cut relation between the rate of urine flow and that of sodium loss is apparent. Of the individual solutes only the apparently greater sodium loss during loading with glucose and creatinine is worth noting. Figure 1B, on the chloride excretion, from which the experiments on loading with sodium chloride have been omitted, presents a similar pattern. Noteworthy is the low excretion rate of chloride in the experiments on loading with sodium sulfate, a circumstance which has been commented on in the preceding section. A higher-than-average chloride loss is suggested with creatinine loading. In figure 1C are presented the potassium losses. Excepting the experiments on loading with sodium salts, all of which show increased excretion, they demonstrate independence to a large extent of the excretion of potassium from the rate of urine flow. The sweeping out of potassium by administration of sodium salts is illustrated clearly. A constant potassium clearance in water diuresis at rates of urine flow exceeding 1 cc/min. has been demonstrated by other workers (7). The relative constancy of the potassium excretion in osmotic diuresis other than that produced by sodium salts would suggest that an increase of osmolarity of the body fluids alone need not lead to marked losses of intracellular potassium as has been suggested on the basis of observations in dehydrated man and animals (8). Figure 1D presents the data on phosphate excretion. Their pattern on the whole is similar to that of the potassium values, although an upward trend with increasing urine flow is suggested. The outstanding exception refers to the experiments on loading with para-aminohippurate in which a distinct increase in the excretion of phosphate occurred. This finding raises interesting speculations with regard to a possible competitive relation between tubular processes concerned with para-aminohippurate excretion on the one hand and reabsorption of phosphate on the other. A constant rate of phosphate excretion has been observed repeatedly in water diuresis (9) but a moderate increase was found in dogs following injection of hypertonic salt solutions (10). The relative constancy of the excretion of potassium and phosphate over a wide range of urine flows is in marked contrast with the proportionality between excretion and flow for sodium and chloride. Among the various factors which may be invoked to explain this circumstance, the osmotic work involved may be cited. With increasing rates of flow the difference in concentrations

between urine and plasma for Na and Cl tends to become greater while it diminishes for K and phosphate. Consequently with increasing urine flow the renal work required to conserve the composition of the body fluids increases for the former and

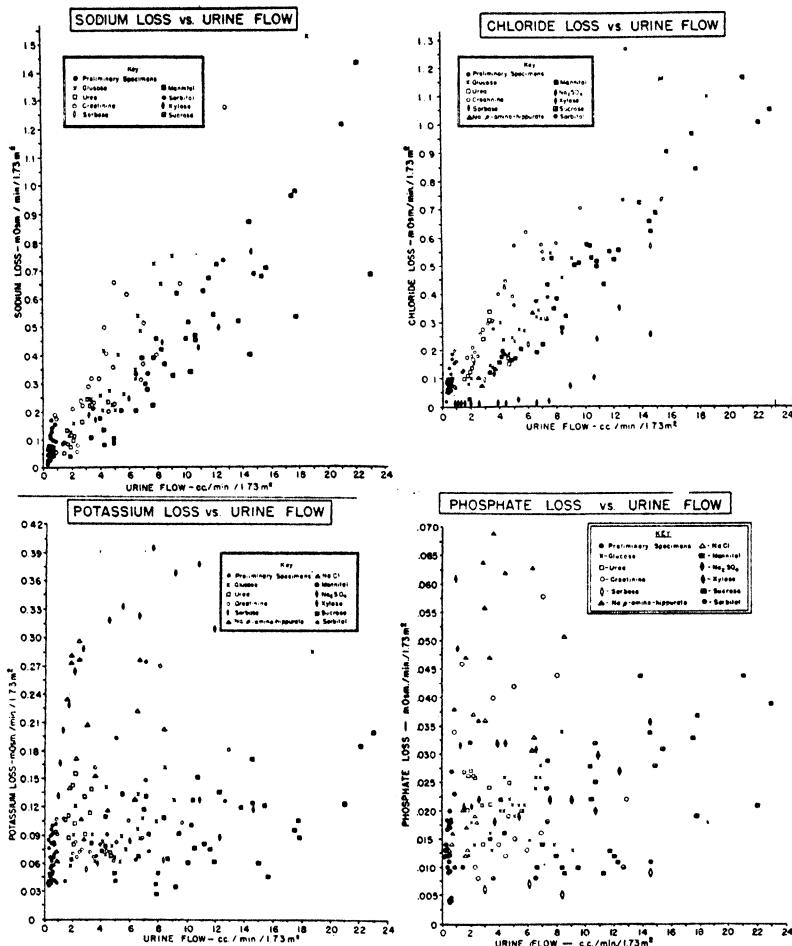


Fig. 1 A, B (upper left and right); C, D (lower left and right). URINARY LOSS OF SODIUM, CHLORIDE, POTASSIUM AND PHOSPHATE DURING OSMOTIC DIURESES. The electrolytes in m.Osm/min. 1.73 M are plotted against the rate of urine flow in cc/min. All experiments with sodium salts are omitted from the sodium graph and those with sodium chloride from the chloride graph.

decreases for the latter ions. The urinary loss with diuresis of sodium and chloride may be a measure of the inefficiency of the renal regulation.

#### *Renal Work in Osmotic Diuresis*

This section of the paper deals with the ideal renal work involved in the process of urine formation under the conditions of osmotic diuresis. It may be well to state

clearly at the outset the limitations and uncertainties of the approach adopted. The general justification for the consideration of the renal work is the basic circumstance that energy is required to produce a given concentration difference between urine and plasma. In the absence of detailed knowledge concerning the exact pathway of urine formation and the metabolic efficiencies of the tubular processes engaged in the elaboration of urine, a minimal value for the renal work is given by the energy thermodynamically required to produce the observed concentration differences. The work is calculated on the assumption of an idealized single-stage process of urine formation conducted in an energetically reversible manner at 100 per cent efficiency. These basic premises are so far removed from the actual conditions of renal function as to raise considerable doubt concerning the biological significance of the calculated ideal work value. Some of the objections may be mentioned. 1) The concept of a single-stage process of urine formation is contrary to the known facts of renal physiology. 2) The assumption of complete efficiency is certainly invalid for any biological system. Actually, data in the literature (11-13) based on the oxygen consumption of the kidney, suggest an efficiency of only 1-2 per cent for the kidney. Furthermore it would be surprising that various types of renal processes, say those involving reabsorption of glucose and of electrolytes, should have the same energetic efficiency. 3) It is by no means certain that the basic assumption of the renal work as that of a chemical concentration cell for each individual solute is valid for all renal processes involving change in the osmolarity of the tubular fluid. In a later section reasons will be cited to doubt this premise for the work in the distal tubule. A number of other equally valid objections may be cited, all of which would suggest that there need be little or no connection between a calculated ideal work value and the actual expenditure of energy by the kidney. Nevertheless, with these reservations in mind, it appeared of interest to explore the possibility that the calculated work during osmotic diuresis would assume a pattern of biological significance and reflect in some manner the actual effective energy expenditure of the kidney, i.e. the portion available for the performance of external work. It appeared conceivable that during solute loading the useful energies of the kidney become engaged to a maximal extent. If so, a measure of the overall work capacity of the kidney would be obtained. Such a maximum would be defined experimentally by a failure to produce a greater work value by increase of either plasma level of loading solute, or urine flow, or urinary load. If such a maximum were discernible further questions would be in order: Is it the same or different for various solutes? How do the individual work values of different solutes contribute to the total? What conditions determine its occurrence? Further questions, not to be taken up here, refer to its possible clinical application. In the following section the ideal work will be presented first as an idealized single-stage process, both as to its total value and to its distribution among different solutes, and secondly subdivided as to proximal and distal urine formation based on assumptions to be discussed later.

*Ideal total work.* The ideal total work is presented for 30 periods on 8 loading solutes in the last column of table 2; and for all 11 solutes, plotted against the rate of urine flow, in figure 2. It may be seen that the work, after first rising with increasing flows, reaches a plateau at a value of about 4.0 cal/min. A more exact analysis of the relation between urine flow and work was attempted in several directions. For a

TABLE 2. IDEAL RENAL WORK DURING OSMOTIC DIURESIS<sup>1</sup>  
Values are for periods listed in table 1; work values are expressed as cal/min/1.73 M<sup>2</sup>

C. B.		UREA							
SPECIMEN NO.	URINE VOL., CC/MIN/1.73 M <sup>2</sup>	UREA	Na	K	Cl	PO <sub>4</sub> <sup>2</sup>	SO <sub>4</sub> <sup>2</sup>	GLUCOSE AND HCO <sub>3</sub> <sup>4</sup>	TOTAL
P-2	0.50	.53	.01	.06	.01	.02	.02	.01	.66
<i>I.V. injection urea, 1385 m.Osm/1.73 M<sup>2</sup></i>									
2	4.58	2.29	.13	.01	.09	0	0	.07	2.59
3	4.61	2.42	.17	.02	.11	.01	0	.07	2.80
G. P.		CREATININE							
SPECIMEN NO.	URINE VOL., CC/MIN/1.73 M <sup>2</sup>	CREATININE	UREA	Na	K	Cl	PO <sub>4</sub> <sup>2</sup>	SO <sub>4</sub> <sup>2</sup>	GLUCOSE AND HCO <sub>3</sub> <sup>4</sup>
P-1	0.66		.60	.01	.11	0	.04	.02	.01
<i>I.V. injection creatinine, 144 m.Osm/1.73 M<sup>2</sup></i>									
3	6.97	2.66	1.26	.19	.20	.02	.03	.01	.11
4	3.51	1.63	.91	.08	.12	0	.02	.01	.05
<i>I.V. injection creatinine, 218 m.Osm/1.73 M<sup>2</sup></i>									
5	7.96	2.54	.58	.24	.16	.02	.01	.01	.12
6	4.97	2.27	.69	.16	.13	.02	.02	.01	.08
W. H.		NaCl							
SPECIMEN NO.	URINE VOL., CC/MIN/1.73 M <sup>2</sup>	Na	Cl	UREA	K	PO <sub>4</sub> <sup>2</sup>	SO <sub>4</sub>	GLUCOSE AND HCO <sub>3</sub> <sup>4</sup>	TOTAL
<i>I.V. injection NaCl, 1052 m.Osm/1.73 M<sup>2</sup></i>									
1	8.36	.12	.23	.74	.13	.02	.01	.13	1.38
2	6.34	.07	.14	.44 <sup>b</sup>	.16	.01	0	.10	.92
3	6.34	.05	.15	.45	.26	.01	0	.10	1.02
L. R.		Na <sub>2</sub> SO <sub>4</sub>							
SPECIMEN NO.	URINE VOL., CC/MIN/1.73 M <sup>2</sup>	SO <sub>4</sub>	Na	UREA	K	Cl	PO <sub>4</sub> <sup>2</sup>	GLUCOSE AND HCO <sub>3</sub> <sup>4</sup>	TOTAL
<i>I.V. injection Na<sub>2</sub>SO<sub>4</sub>, 758 m.Osm/1.73 M<sup>2</sup></i>									
1	8.94	1.96	.40	.40	.23	.42	0	.14	3.55
2	5.31	1.16	.32	.42	.28	.29	.01	.08	2.56
<i>I.V. injection Na<sub>2</sub>SO<sub>4</sub>, 506 m.Osm/1.73 M<sup>2</sup></i>									
3	10.6	2.14	.43	.29	.20	.48	0	.16	3.70
4	7.45	1.58	.44	.43	.32	.45 <sup>b</sup>	0	.11	3.33

## RAPOPORT, WEST AND BRODSKY

TABLE 2.—Continued  
NA P-AMINOHIPPURATE

A. R.

SPECIMEN NO.	URINE VOL., CC/MIN/1.73 $M^2$	PAH	Na	UREA	K	Cl	$PO_4^2$	GLUCOSE AND $HCO_3^-$	TOTAL
<i>I.V. injection, NaPAH 113 m.Osm/1.73 M<sup>2</sup></i>									
2	2.77	1.40	.04	.33	.04	.07	.08	.04	2.00
<i>I.V. injection, NaPAH 220 m.Osm/1.73 M<sup>2</sup></i>									
4	4.35	1.54	.04	.43	.05	.07	.05	.07	2.25
5	3.52	1.50	.03	.45	.11	.05	.07	.05	2.26

A. R.

SPECIMEN NO.	URINE VOL., CC/MIN/1.73 $M^2$	MANNITOL	UREA	Na	K	Cl	$PO_4^2$	GLUCOSE AND $HCO_3^-$	TOTAL
<i>I.V. injection mannitol, 467 m.Osm/1.73 M<sup>2</sup></i>									
1	10.3	3.15	.25	.42	.02	.09	0	.16	4.09
2	7.29	2.82	.38	.27	.02	.05	.01	.11	3.66
<i>I.V. injection mannitol, 467 m.Osm/1.73 M<sup>2</sup></i>									
3	17.7	3.53	.17	.71	0	.16	0	.27	4.84
4	13.7	3.19	.12	.47	.01	.11	0	.21	4.11
<i>I.V. injection mannitol, 467 m.Osm/1.73 M<sup>2</sup></i>									
5	22.8	3.58	.10	.87	.02	.23	0	.35	5.15
6	14.4	3.37	.17	.60	.03	.14	0	.22	4.53

D. W.

SPECIMEN NO.	URINE VOL., CC/MIN/1.73 $M^2$	SUCROSE	UREA	Na	K	Cl	$PO_4^2$	GLUCOSE AND $HCO_3^-$	TOTAL
<i>I.V. injection sucrose, 392 m.Osm/1.73 M<sup>2</sup></i>									
1	14.8	2.72	.20	.41	0	.18	0	.23	3.74
2	10.6	2.60	.39	.32	.01	.13	0	.16	3.61
<i>I.V. injection sucrose, 196 m.Osm/1.73 M<sup>2</sup></i>									
3	15.3	2.84	.29	.47	.01	.19	0	.24	4.04
4	10.6	2.83	.25	.34	.04	.14	0	.16	3.76

L. D.

TABLE 2.—Continued

## GLUCOSE

SPECIMEN NO.	URINE VOL., CC./MIN./1.73 M <sup>2</sup>	GLUCOSE	UREA	Na	K	Cl	PO <sub>4</sub> <sup>2</sup>	SO <sub>4</sub> <sup>2</sup>	HCO <sub>3</sub> <sup>4</sup>	TOTAL
P-2	0.86	.57	.38	0	.04	0	.01	.01	.01	1.02

Glucose, oral, 366 m.Osm/1.73 M<sup>2</sup>  
I.V. injection glucose, 256 m.Osm/1.73 M<sup>2</sup>

1	9.04	2.49	.42	.07	.02	.07	0	0	.11	3.18
2	7.79	2.17	.40	.04	.02	.03	0	0	.10	2.76
I.V. injection glucose, 476 m.Osm/1.73 M <sup>2</sup>										
3	18.5	3.11	.18	.13	.06	.13	0	0	.23	3.84

<sup>1</sup> The work was calculated according to equation 1. For this purpose the plasma concentrations of the loading solutes at the midpoint of the urine collection periods were determined by graphic interpolations from the slope of the disappearance curves. All plasma values were multiplied by 1.05 to give an assumed value for the concentration in the glomerular filtrate.

<sup>2</sup> Phosphate, plasma concentration assumed in m.Osm/l.; W. H., NaCl, 1.3; C. B., urea, 1.4; G. P., creatinine periods 3 and 4, 1.6, periods 6, 1.7; L. R., Na<sub>2</sub>SO<sub>4</sub>, periods 2 and 3, 1.2; A. R., NaPAH, periods 2 and 5, 1.3; D. W., sucrose, periods 2 and 3, 1.5; L. D., glucose, periods 2 and 3, 0.9.

<sup>3</sup> Sulfate, plasma concentration assumed in all cases where not determined, 1. m.Osm/l.

<sup>4</sup> The work is calculated on the assumption of a value for the sum of glucose and HCO<sub>3</sub> of 25 m.Osm/l in the plasma and zero in the urine. In the case of the glucose experiment, a plasma concentration of 20 m.Osm/l. was assumed for HCO<sub>3</sub> alone.

<sup>5</sup> Plasma concentration assumed 102.0 m.Osm/l.

<sup>6</sup> Plasma concentration assumed 4.8 m.Osm/l.

statistical estimate of the maximum work value 31 experimental periods with urine flows in excess of 10 cc/min. were considered. Three aberrantly low mannitol values observed during periods of rapid change of urine flow, were omitted. The remaining 28 periods yielded a mean value for work of  $3.95 \pm 0.09$  cal/min., with a standard deviation of 0.49 cal/min. Calculation on this material of the linear regression of work on urine flow gave a small but significant regression coefficient of  $0.064 \pm 0.023$  cal/min/cc. of urine flow. The probability that such a regression could have arisen by chance alone was 0.01. Tests for association were carried out on the mannitol experiments separately over a wider range of flows, in order to eliminate any effect arising from the multitude of loading solutes employed. They led to essentially identical results as those when all solutes were considered. Statistically, then, the rate of urine flow even in the range of high values exerted a slight effect on the magnitude of the work value. In the following discussion this subordinate effect for the most part will be disregarded.

All solutes are represented in the maximum range of work values excepting NaCl, urea and NaPAH. The fact that NaPAH fell short of the maximum is not surprising in view of the rather small amount administered. In the case of NaCl and urea, which were given in large dosage, a different explanation applies. Given a ceiling of urinary osmolarity, dependent on urinary flow (1), the initially high plasma

concentration of sodium and chloride limits the U/P ratio and therefore the possible work value below the maximum. In the case of urea the extensive tubular absorption in causing a rapid rise of the plasma concentration produces the same effect during loading.

The assumption of a maximum of work demands the determination of the independence of the work value not only from the rate of urine flow, but also from urinary

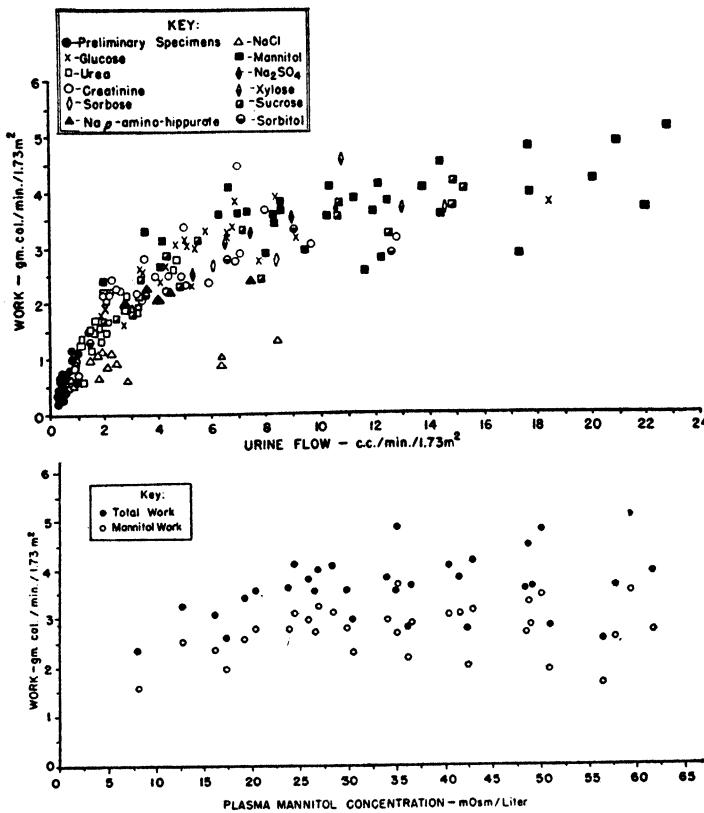


Fig. 2. IDEAL TOTAL WORK vs. urine flow

Fig. 3. IDEAL TOTAL AND MANNITOL WORK vs. plasma concentration of mannitol.

load and plasma level of loading solutes. A plot of work versus urinary load is in all respects similar to that versus urinary flow, shown in figure 2, and is therefore omitted. It also indicated on statistical analysis of the experimental periods at urine flows in excess of 10 cc/min. a slight but significant dependence of work on urinary load, with a regression coefficient of  $0.19 \pm 0.05$  cal/min/unit load. The relation of work to the plasma level is exemplified by the data on mannitol loading summarized in figure 3. It contains a plot of total and of mannitol work versus plasma level of mannitol. An independence of the total work value from the plasma level over a

range from 20 to 62 m. Osm/l. is apparent. Statistical analysis of the data corroborates this impression. The linear regression of work on plasma level of mannitol yielded a non-significant coefficient of less than 0.005 cal/min/m. Osm/l. of plasma level. The probability of its occurrence by chance alone was 0.5. Thus, in this case, even a slight dependence of work on the plasma level, such as noticed previously with respect to urine flow and load, is absent.

In figure 4 is presented the work per cc. of urine elaborated, plotted versus the rate of urine flow. It is seen that this work decreased sharply at first and more slowly later. On the whole, the pattern of the graph is strongly reminiscent of that of the plot of urine osmolarity versus urine flow (2). Theoretically, once renal work reaches its maximum,  $W/\text{cc.} \times \text{volume}$  becomes constant and a strict reciprocal relation between the work per cc. and urine flow obtains.

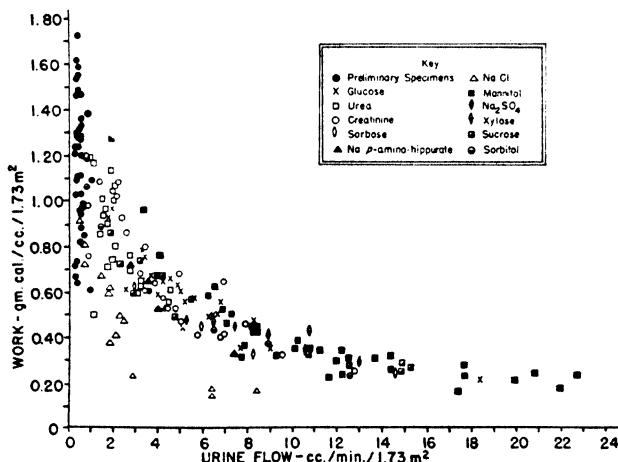


FIG. 4. WORK PER CC. OF URINE elaborated vs. rate of urine flow

An important question refers to the relation of the work to the duration of osmotic diuresis. *A priori*, 3 possibilities are apparent: 1) independence of the work value from the duration of diuresis; 2) exhaustion of the renal mechanism during continuous loading; and 3) a lag with increased efficiency during prolonged loading. Some of the relations observed are plotted in figure 5, which shows progressive changes in the relation between total work and flow during four representative experiments involving urea, sodium chloride, mannitol, and creatinine. Although there is no indication of any effect of repeated loading on the maximum work value, there is clear evidence of hysteresis, with the work during de-loading proceeding at a much higher level than during loading. The level during de-loading is close to the maximum.

The distribution of the work values among the different solutes may be considered next. Table 2 contains a compilation of the ideal work values during osmotic diuresis for the periods presented in table 1. Included are the individual values of the solutes determined, an assumed value for bicarbonate and glucose, and a calculated

total value. It is seen that in most instances, the loading solute accounted for the bulk of the renal work. Its contribution amounted to more than 85 per cent in the case of urea, and to more than 75 per cent in the mannitol, sucrose and glucose experiments. Similar high percentages were calculated for all loading solutes except for the sodium salts. With sodium para-aminohippurate, the anion alone accounted for about 70 per cent of the ideal work, while the sodium contribution was negligible. It was equally small in the sodium chloride experiment while the chloride contribution, although considerably greater, was only 0.23 cal/min. The highest value of sodium work was observed with sodium sulfate, where a value of 0.44 cal/min. was

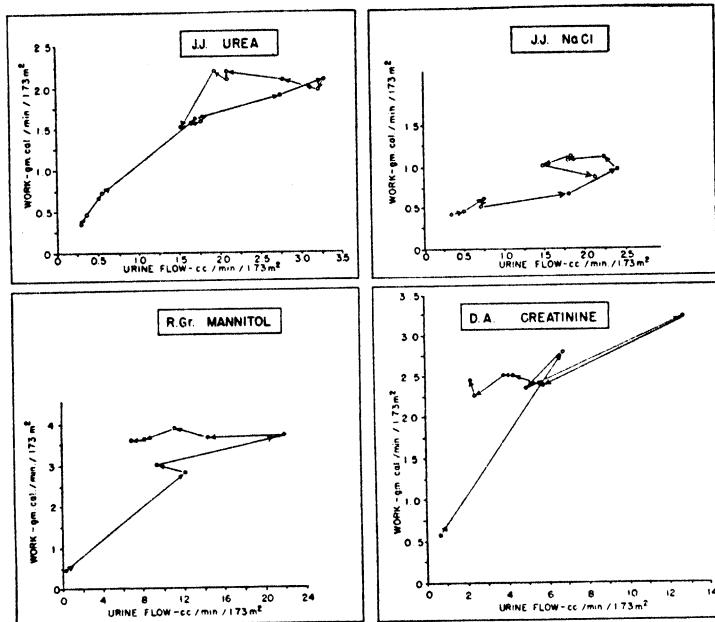


Fig. 5. SEQUENCE OF RELATIONS between work and urine flow during osmotic diuresis. Arrows indicate the order of the observations.

calculated, still only about 10 per cent of the total work. In that experiment the sulfate contribution accounted for approximately 50 per cent of the work. In experiments other than those involving sodium salts the sodium work tended to increase as the urine flow rose, while the reverse was true for potassium. Both of these trends are as expected, since with increasing flow the concentration difference between plasma and urine increases for sodium and decreases for potassium. Chloride on the whole followed the pattern of the sodium, and urea, phosphate and sulfate that of potassium. The principal exception refers to the potassium work during loading with sodium salts. Under these conditions its work value rose during diuresis. As a matter of fact the potassium contribution in one sodium chloride experiment accounted for 40 per cent of the total work. A relatively large value of the chloride work during

loading with sodium sulfate, a reflection of the phenomenon of depressed excretion discussed in the preceding section, is worthy of note. Also of interest is a high value of the urea work during one experiment with creatinine loading. But in two other experiments its contribution was not so great.

*Osmotic work in proximal and distal tubule. Theoretical considerations.* The calculations heretofore described have referred to the idealized direct process of urine formation from the glomerular filtrate. Actually there is good reason to believe that the concentration of a solute may change in opposing directions in different parts of the nephron. It is extremely unlikely that such changes are energetically reversible, i.e., that the energy expended by the cells in one portion of the tubule in producing a given concentration difference may be regained by those in another portion if the tubular fluid returns to a previous concentration. Therefore the work calculated for the different portions of the tubule, if the actual concentration changes in each portion were known, would be greater than that calculated on the assumption of a single-stage process. The knowledge of the actual stages of the process of urine formation is still too fragmentary to permit the calculation of the work for each segment of the tubule. However, one over-all division of the process of urine formation has become generally accepted and may be profitably discussed. It is assumed that the process of water reabsorption proceeds in two stages: The first in the proximal tubule, at a constant osmolarity, isotonic with plasma, but with a changing distribution of solutes; and the second distally, with the production of a hypertonic urine (14). Previously (1, 2) a dependence of urine flow and osmolarity on solute load which was independent of the composition of the urine has been demonstrated. The character of this relation could be explained on the assumption of a constancy of the solute load in the distal tubule, with reabsorption of water alone taking place. The process of water reabsorption could be expressed in terms of load as follows:

$$R_{pr} = GFR - \frac{\text{Load}}{O_p} \quad \text{and} \quad R_d = \frac{\text{Load}}{O_p} - V \quad (2)$$

where  $R_{pr}$  and  $R_d$  refer to the amounts of water reabsorbed in the proximal and distal tubules respectively,  $O_p$  is the total osmolarity of plasma, assumed to be equal to that of the fluid in the proximal tubule, GFR is the amount of glomerular filtrate and  $V$  of urine. On the basis of these relationships calculation of the concentration of the solutes and the volume of fluid at the dividing point of proximal and distal tubule, and therefore a separate consideration of the work in the proximal and the distal tubule, become feasible. The concentration of a solute, as it leaves the proximal tubule,  $U_{pr}$ , is given as

$$U_{pr} = \frac{U \times O_p}{O_u} \quad (3)$$

where  $U$  refers to concentration of the solute in the urine,  $O_u$  is the osmolarity of urine and  $O_p$  that of the fluid in the proximal tubule, assumed to be isotonic with plasma. The volume of the fluid as it leaves the proximal tubule,  $V_{pr}$ , is given as

$$V_{pr} = \frac{V \times O_u}{O_p} \quad (4)$$

where  $V$  is the volume of the urine and the other symbols have the same meaning as before. With these data on hand the work in the proximal tubule for a given solute may be calculated on the basis of *equation 1*. The total work in the proximal tubule is given by the sum of the work values for all individual solutes.

Two alternative processes may be considered for the work in the distal tubule: 1) a process similar in nature to that in the proximal tubule. In essence, this would mean that in the distal tubule, as proximally, work is done on each solute independently, varying for each constituent with its specific concentration ratio. The total work, varying according to the composition of the solute load, is given by summation of the individual work values. The situation would be comparable to the calculation of the energy output of a series of concentration cells of different chemical composition. This assumption may be designated as the 'chemical' theory of distal work. 2) It is not the chemical composition but only the osmolarity of urine and plasma that determine the necessary work. The situation would be analogous to the work process across a semi-permeable membrane, which is permeable to water alone but not to any solute particle. The energy of such a process would be given by the same equation as previously employed, save that the total osmolarities of plasma and urine are used instead of the individual concentrations of the several solutes. The factors determining total work in the distal tubule would then be the ratio of the urine and plasma osmolarities and the urine flow, independent of the chemical composition of the solute load. This hypothesis may be called the 'osmotic' theory for the work in the distal tubule. In general the total work value calculated in this way will be smaller than that based on the 'chemical' theory.

At present a decision between these two theories is not at hand. But two circumstances which are difficult to explain by the 'chemical' theory, but are predicted by the 'osmotic' theory, are apparent: 1) the existence of a maximal osmotic ceiling for urine at minimal flows, which is independent of its composition; and 2) the dependence during osmotic diuresis of urine flow and osmolarity on load alone, independent of the nature of the loading solute.

One implication of the 'osmotic' theory may be mentioned. It would suggest that water alone moves across the distal tubular wall, either in the process of reabsorption for the production of hypertonic urine, or secretion during water diuresis. The function of the antidiuretic hormone may well be linked up with the energetics of this process.

In figure 6 is depicted a graph of the relation of work and load in the distal tubule on the basis of the 'osmotic' theory. It is constructed on the assumption of a constant value of 0.33 Osm/l. for the osmolarity of the fluid in the proximal tubule. It may be seen that the distal tubular work reaches a maximum at a load of about 4 m. Osm/min., corresponding to flows of 8 cc/min. With further increase in load and flooding of the distal tubule the work delivered actually decreases. The total work in the distal tubule even at its maximum represents only a small fraction, approximately 12 per cent, of the proximal work.

*Application to present experiments.* It appears of great interest to apply the concepts discussed to the experimental material at hand.

Before engaging in a detailed scrutiny of the data one can predict that this form

of calculation will result in a decreased work contribution for those solutes that are little or not reabsorbed in the tubule. On the other hand the work will increase for

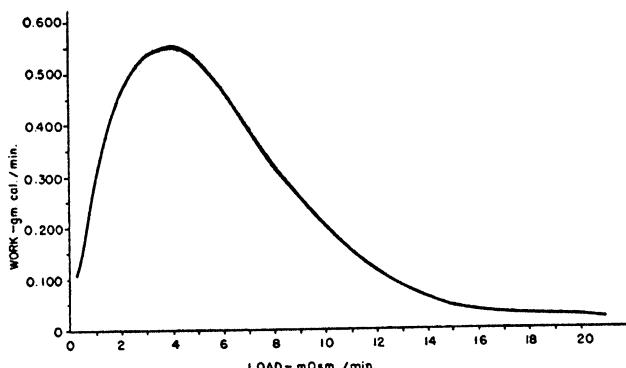


Fig. 6. WORK VS. LOAD IN THE DISTAL TUBULE, calculated on the assumption of an osmolarity of 0.33 Osm/l. for the fluid leaving the proximal tubule.

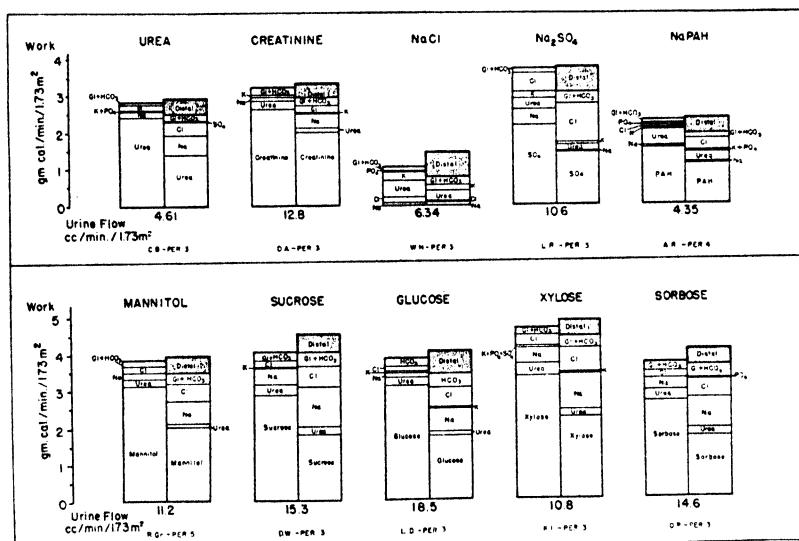


FIG. 7. COMPARISON OF IDEAL TOTAL WORK with proximal and distal work for 10 loading solutes. The work values for the individual solutes are graphed in an additive manner in bar diagrams. The proximal work is indicated by a shaded area. Periods of maximal work were chosen for the comparisons.

the solutes that tend to be reabsorbed extensively. In figure 7 are presented illustrative comparisons between the ideal total work, and the proximal and distal work for 10 of the loading solutes, sorbose alone having been omitted. Periods of greatest work for each loading solute were chosen for the presentation.

For the osmotic work in the proximal tubule, the volume and solute concen-

trations of the fluid at the junction of the proximal and distal segments of the tubule were calculated by *equations 3* and *4*. For the calculation the value  $O_p$ , the osmolarity of the fluid in the proximal tubule, was calculated for each period as described in the footnote of table 1. The method of calculating the urine osmolarity,  $O_u$ , has been described previously (2). The value of  $V_{pr}$ , the volume of the fluid as it leaves the proximal tubule, and  $U_{pr}$ , the concentration of a solute in the fluid, were substituted for  $V$  and  $U$  respectively in *equation 1*. The total work in the proximal tubule was the sum of the works for each solute.

For the calculation of the osmotic work in the distal tubule the osmolarity of urine,  $O_u$ , and the osmolarity of plasma,  $O_p$ , were substituted for  $U$  and  $P$  in *equation 1*.

The outstanding points in the comparison are as follows: 1) the total work value on the whole is the same, whether it is calculated as a single-stage chemical process (ideal total work) or is subdivided into proximal and distal contributions; 2) the

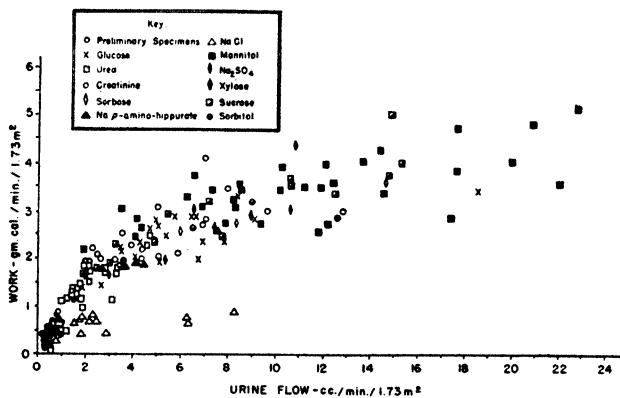


Fig. 8. PROXIMAL WORK VS. URINE FLOW

distal work is always a small fraction of the total which reaches a maximum at urine flows of about 8 cc. per minute; 3) although the proximal work value approximates the ideal total work, the distribution of the work among individual solutes differs considerably. The variations are in the predicted direction. In all loading experiments not involving sodium salts, the sodium and the chloride work assumes a prominent place, while the work of the loading solute decreases.

In figure 8 a plot of the proximal work versus urine flow for all solutes is presented. It is evident that its pattern is entirely similar to that of figure 2 which depicts the relation of the ideal total work versus urine flow. A maximum of the same magnitude is apparent. The conclusion appears justified that the ideal total work in effect represents the proximal work value.

#### DISCUSSION

The following discussion will deal with 2 items: 1) a schematic presentation of the inter-connection between the urinary flow-load relation on the one hand, and on the other the renal work; and 2) the relations between the calculated work and the actual energy metabolism of the kidney.

1) *Theoretical work for a single solute.* The general equation for renal work (1) may be expressed more suitably for this discussion as

$$W = RT \text{ load} (\ln U/P + P/U - 1) \quad (5)$$

where  $W$  is the work,  $V$  the urinary volume,  $U$  the urinary and  $P$  the plasma concentration of a given solute, and load is equal to  $U \times V$ , has 3, *a priori*, independent variables. It contains no inherent limitations for the magnitude of any of the variables, or the work value. The experimental fact that urine flow and urinary osmolarity are not independent of each other, but are a function of load, may be used to reduce the number of variables to 2, if the simplifying assumption is made that a single solute accounts for the entire osmolarity of urine. Then  $U$  for man may be expressed (2) in terms of load as

$$U = 0.847 e^{-0.21 \text{ load}} + 0.33. \quad (6)$$

Substitution of this expression for  $U$  in *equation 2* permits one to define work in terms of load and plasma concentration alone. Actually, even under conditions of maximal work, as shown in the experimental part, the loading solute accounts for only about 80 per cent of the urine osmolarity. The simplification employed qualifies the quantitative applicability of the derivations but does not modify to an important extent the significance of the patterns.

Another experimental datum, the existence of a biological maximum of work of 4.0 cal./min. for man, serves to delimit the biologically possible magnitude of the work value.

Thus it is possible to give a schematic portrayal of the inter-dependence of  $W$ , load, and  $P$  in the biologic range, incorporating the determined flow-load relations on the basis of the assumption of a single urinary solute. A complete presentation would require a three-dimensional grid. Here the inter-relations are presented in the form of 3 graphs, in each of which 2 of the factors are plotted as continuous variables on the ordinates, and the third one is fixed at a series of values.

In figure 9 work is plotted against load for a series of different assumed plasma levels of solute. Certain values of urine flow corresponding to the given loads are also indicated on the abscissa. A dotted line indicates the position of the approximate biological maximum of renal work for man. It may be seen that for low plasma levels the work rises steeply with increasing load. As the plasma level increases the work curves describe increasingly flat trajectories to reach the biological maximum at increasingly high loads. Finally at plasma levels between 100 and 150 the biological maximum is unattainable within the limits of biologically possible urine flows. It is evident that once maximum work has been reached further loading will result only in a horizontal displacement of the work value, although plasma levels and urine loads increase. It is also of interest to view the values for plasma and urine which are coincident with maximum work from the standpoint of clearances. It may be seen that for a plasma level of 1 m. Osm/l. maximum work is reached at a load of 1 m. Osm/min. This corresponds to a clearance of 1000 cc. of plasma/min., obviously an impossible value. With a clearance of 100 cc., i.e., approximately at the level of glomerular filtration, maximum work is reached for a

plasma level of 40 with a load of 4 m. Osm/min. These figures are of the same order of magnitude as has been actually observed under conditions of loading in mannitol experiments.

In figure 10 work is plotted versus plasma level for different loads. The plasma values are arranged in a logarithmic manner. This graph brings out the fall in work with increasing plasma levels and constant load, an aspect which has been stressed by Newburgh (15). It also illuminates the circumstance that for high plasma levels increase of load leads to only minor change in the work value. It is clear that the biological maximum is unattainable for high plasma values.

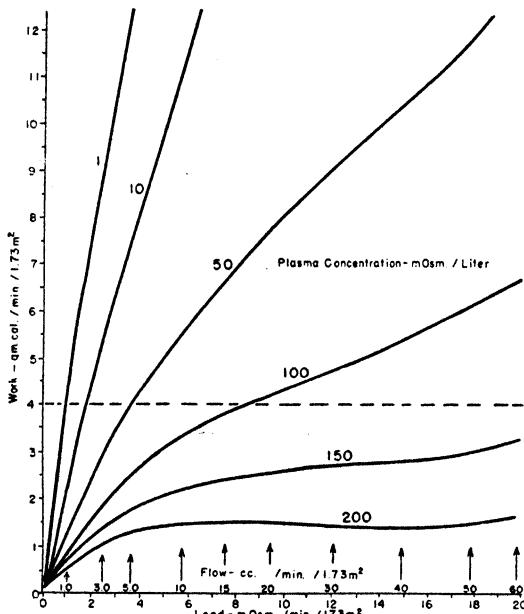


Fig. 9. WORK VS. LOAD for different plasma concentrations of the loading solute, calculated on the assumption of a single urinary solute.

In figure 11 is presented the third aspect of the relations. Load is plotted versus plasma level for a series of work values. Both variables are plotted in a logarithmic manner. This graph brings out the relationship between plasma and urine for constant work. The steep final portions of the graphs deserve emphasis. They illustrate the increasing inefficiency of increasing loads to produce increased work at high plasma values.

The theoretical impossibility to reach the biological maximum of renal work at high plasma levels within the known limits of the load-flow relations has interesting implications. It serves to explain the inability to produce maximal work by loading with sodium or chloride, solutes normally occurring in high concentrations in plasma. It indicates a similar limitation for urea, which because of its tubular reabsorption,

and low clearance, rapidly reaches high values in plasma during loading. Finally it applies to pathologic conditions, where a given work value may be maintained, with increasing loads cleared, at the expense of high plasma concentrations.

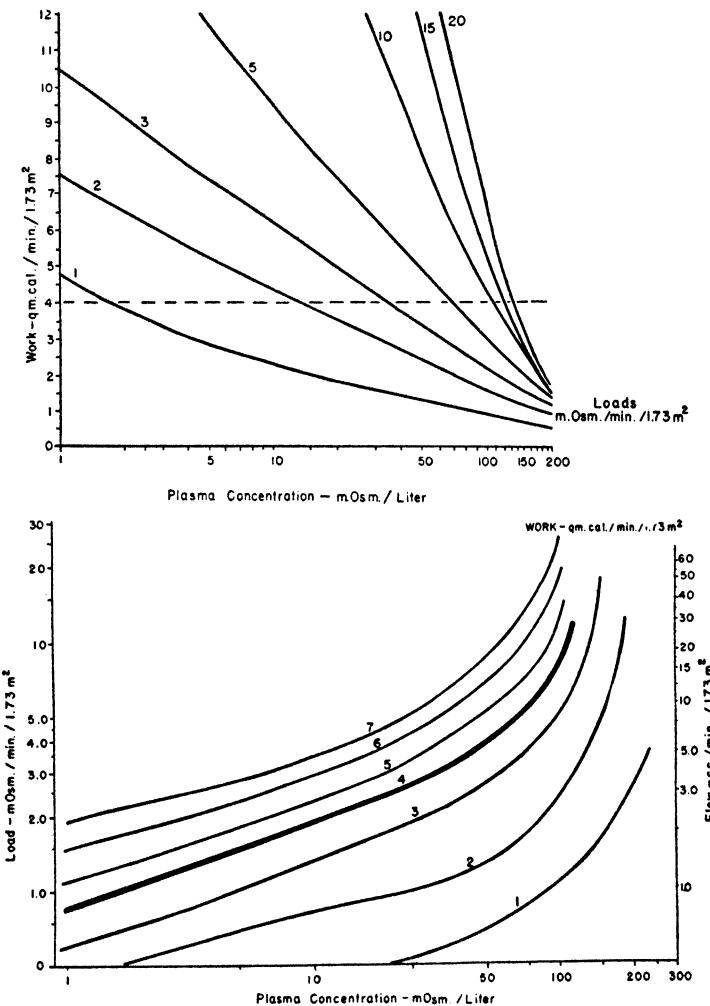


Fig. 10. WORK VS. PLASMA CONCENTRATION for different loads

Fig. 11. LOAD VS. PLASMA CONCENTRATION for different work values

2) *Calculated work and energy metabolism.* The oxygen consumption of the kidneys of man under 'resting' conditions has been estimated by Bradley and Halperin (6) on a small group of subjects to be about 10 cc/min. with a range of  $\pm 4.0$  cc. This corresponds to about 0.03 cc. of oxygen per gm. of tissue, if an average weight of 300

gm. is assumed for the kidneys of man. In dogs, an average value of 0.07–0.08 cc. per gm. (12, 17) or higher (18, 19) and an even greater variation has been reported. Assuming a respiratory quotient of 1.0 so that 1 cc. of oxygen corresponds to 5.0 cal., and as a minimum figure for the oxygen consumption, the resting value of 10 cc/min., a total energy of 50 cal/min. would be available to the kidneys of man. It has been shown that the thermodynamic work of the hydropenic kidney under resting conditions accounts for about 0.6 cal/min. (3), a little more than 1 per cent of the total energy available. This proportion is of the same order of magnitude as has been measured directly in animals (11, 12) and estimated indirectly for man (20). During osmotic diuresis, the thermodynamic work rises to an average maximum level of 4.0 cal/min., accounting at the most for 8 per cent of the total energy available, and probably for less. An increase in efficiency during diuresis, i.e., a relatively greater increase in the work compared with the oxygen consumption has previously been reported for the isolated kidney of dogs (13). The effect of osmotic diuresis on the oxygen consumption has been variously reported. Whereas early workers described an increase during diuresis produced by either urea or sodium sulfate (21), later observers failed to find any correlation between urine flow or thermodynamic work and oxygen consumption (12, 13, 17, 22). On the other hand a positive correlation between oxygen consumption and blood flow was described (12, 17). Van Slyke *et al.* (17) suggested that both blood flow and oxygen consumption increased in response to the metabolic demands of the kidney and concluded: "The overwhelmingly greater part of the energy must be utilized by the kidney for its own internal cellular processes not related to the external work which the organ is performing."

Although the conditions of the experiments cited differed considerably from those obtaining in our study, since all previous workers secured a copious urine flow by hydration of their subjects or experimental animals, their main conclusion probably applies, namely that thermodynamic work accounts for only a small fraction of the total energy, and that there is little relation between the external work of the kidney and its metabolic demands. On the other hand the existence of a reproducible maximum of thermodynamic work suggests that the calculated values reflect some aspect of the energy metabolism of the kidney, perhaps its actual effective external energy expenditure. Whether this portion of the renal energy is so small as to be obscured by other metabolic needs of the kidney; or whether one is dealing with a diversion of the energies from internal to external use, so that their sum remains constant, cannot be decided at present. The increase in efficiency during diuresis, reported by Eggleton *et al.* (13) perhaps favors the latter hypothesis.

Accepting the premise of the biologic validity of the calculated data, certain implications of a maximum of renal work should be pointed out. In particular, the circumstance that the same maximum was calculated for a variety of solutes, appears surprising in view of the presumably widely differing renal mechanisms engaged.

Considering first the non-electrolytes, the equality of maximal work for glucose, which is reabsorbed to a significant extent in the tubule, and sucrose, which is not, would suggest that the tubular reabsorption process contributes only a minor share to the total work. The work imposed by the fraction escaping reabsorption over-

shadows it by far, and may be quite similar among the various sugars and sugar alcohols. Such an explanation does not satisfy in the case of sodium sulfate loading. A similarity in the efficiency of widely varying renal mechanisms may be proposed, or the existence of a common energy pool from which all renal processes draw. Given a fixed total value of osmotic work capacity the distribution of the renal work among the several processes would then vary according to the type of loading solute. The existence of such a common energy pool, for which various renal mechanisms compete, would betray itself by the fact that although the total work value remains constant the distribution of the work among different solutes may vary with the urine flow. With increasing flow one would expect the contribution of the loading solute to diminish and that of the sodium and chloride to increase. The experimental observations are in agreement with this concept. A further consideration may be advanced. One might reason that under the circumstances of hydropenia considered, with water as the over-all limiting factor the bulk of the work consisted in abstracting water from the loading solutes and preserving the body electrolytes. The work considered in the present experiments may then have neglected other external functions of the kidney.

Another question arises as to whether the maximum found in the hydropenic state describes the over-all osmotic work capacity of the kidney under all conditions. An obvious extension of the inquiry should encompass a study of water diuresis and of the rôle of the anti-diuretic hormone.

#### SUMMARY

The excretion of solutes and the renal work under conditions of osmotic diuresis in man has been presented. Eleven loading solutes were administered in amounts of 500–2000 m. Osm/1.73 M<sup>2</sup> body surface. They were: glucose, sucrose, mannitol, sorbitol, sorbose, xylose, urea, creatinine, sodium para-aminohippurate, sodium sulfate and sodium chloride.

During loading the urine flow increased widely up to 22.8 ml/min. The plasma osmolarity averaged  $330 \pm 30$  m. Osm/l., while the urine osmolarity decreased. The concentration of the loading solute in plasma rose as high as approximately 60 m. Osm/l. in the case of mannitol. In the urine it averaged 330 m. Osm/l. for most loading solutes with wide individual variations. As the urine flow rose the excretion of sodium and chloride rose proportionately, while that of potassium and phosphate tended to remain constant. Sodium salts produced an increased potassium loss, while sulfate decreased the chloride loss.

The calculated ideal osmotic work rose during loading to a maximum of about 4.0 cal/min., a value which was not increased by further augmentation of urine flow or load or plasma level of the loading solute. The same maximum value was reached with all loading solutes studied excepting NaCl, NaPAH and urea. NaPAH was not administered in adequate amounts, but in the case of urea and NaCl, theoretical reasons exist for the failure to reach maximum work.

A subdivision of the renal work in proximal and distal portions, based on the observed flow-load relations of urine has been attempted. It is suggested, that while the proximal tubular work is essentially 'chemical' in nature, depending on the

concentration in plasma and urine of individual solutes, that in the distal tubule is 'osmotic,' depending on the total osmolarity of plasma and urine. The proximal work is closely similar in magnitude to the ideal total work, although the distribution of the work values among the individual solutes differs widely. The distal work is usually a small component of the total work. The relations among the renal work, the plasma concentration and the urinary load under the simplifying assumption of a single urinary solute have been discussed and graphically presented.

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# INULIN SPACE AS A MEASURE OF EXTRACELLULAR FLUID

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**T**HE measurement of the extracellular volume must be made with a substance which is completely distributed in that space but excluded from the intracellular compartment. Ideally this substance should also fulfill the following conditions: 1) fairly rapid and uniform distribution; 2) no formation or destruction in the organism; 3) negligible osmotic effect; 4) slow or measurable elimination from the body; 5) no toxicity, and 6) accurate and easy determination.

The first attempt to measure the extracellular space was made visually in frozen preparations of muscle (1) and the value obtained was 15 per cent of the total weight. The fact that the total quantity of chloride found in muscle, if distributed in that histological space, would be in the same concentration as in the plasma, led to the conclusion that all the chloride was extracellular (2). This assumption was extended to include the whole body, and chloride and consequently sodium were considered to be limited to extracellular distribution (3-9), although it has been recognized that sodium and chloride enter the cells to a variable degree (5, 7, 8, 10-13). Methods using these electrolytes will give volumes of distribution which are variably larger than the actual extracellular space. This conclusion can be extended to bromide which is distributed in the same volume as chloride (14, 15). Sodium thiocyanate, one of the substances most widely used for this purpose, also enters the cells (16, 9, 17, 18). When the above substances were used simultaneously in the dog, the values obtained were: thiocyanate, 35.6 per cent of body weight; radioactive sodium, 27.6 per cent; and radioactive chloride, 24.7 per cent (9).

To avoid the disadvantage of the entrance of electrolytes into the intracellular space other substances were sought to which the cell membrane was impermeable. Attention was focused on the carbohydrates, sucrose (19), mannitol (20, 21), and inulin (13, 22). Sucrose was used despite the disadvantages of a rapid excretion and an incomplete urinary recovery in the dog (23). The recovery of mannitol in urine is also incomplete (24, 25), indicating some utilization.

Inulin has several advantages over any of the above substances. It is not an electrolyte, is lipid insoluble and has a large molecular weight, all circumstances that reduce the probability of its permeating the cellular membrane. It does not penetrate the erythrocyte (26), diffuse through the normal renal tubule (26, 27), or undergo concentration by the liver cells (22, 28). The circumstance that it is rapidly and quantitatively recovered in the urine argues against its being metabolized to any appreciable degree or stored in any tissue (24, 29, 30). Furthermore, it is physiologically inert and exerts negligible osmotic pressure (29, 31). It has the disadvantage of being rapidly excreted by glomerular filtration, which prevents uniform distribution throughout the extracellular space after a single injection. This fact has limited its use, when given by the customary methods, to nephrectomized animals (13, 22), in which the volume of distribution is significantly smaller than the volume of distribution of chloride or thiocyanate.

An equilibration method which permits the use of inulin for the measurement of extracellular fluid in normal animals and man has been reported in a preliminary

Received for publication February 28, 1949.

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paper by the present authors (30). The procedure was devised to overcome the rapid excretion of inulin. The purpose of this paper is to report the complete results obtained with this method and the modifications introduced to minimize the errors arising in its practical application.

#### METHODS

Inulin space determinations were made on normal trained, unanesthetized dogs. The procedure depends on the maintenance of a steady infusion which compensates for excretion and maintains the plasma level constant until a uniform concentration exists throughout the extracellular space. Extracellular equilibrium is more rapidly attained if the infusion is preceded by a priming dose, calculated for each dog according to the desired plasma level and expected volume of distribution. All the experiments have been done with an infusion rate less than 1 cc. per minute so that the extracellular space is not significantly augmented. The constancy of the infusion afforded by the mercury drip method has proved to be adequate and the accuracy of the measurement does not increase with more elaborate means of maintaining constant infusions.

Once equilibrium is established, a blood sample is withdrawn, the bladder is emptied by catheter and rinsed and the infusion simultaneously discontinued. The urine is then collected until the total amount of inulin contained in the body has been excreted, 5 hours in the dog, 12 hours in man (30). The quantity of inulin recovered in milligrams, divided by the plasma concentration in mg/cc. equals the volume of distribution in cubic centimeters.

Prior to the inulin injection a control blood and timed urine sample are obtained for the determination of the corresponding blank corrections (inulinoid plasma blank in mg/cc. and urine inulinoid blank in mg/minute).

Thiocyanate and radioactive sodium ( $\text{Na}^{24}$ ) spaces were determined by the single injection method. Blood samples for the determination of these substances were withdrawn after 1 hour for thiocyanate and after 3 hours for  $\text{Na}^{24}$ . Chemical analyses were made by the method of Harrison (32) for inulin, and Crandall and Anderson (16) for thiocyanate. Radioactive sodium was measured with a Geiger-Müller counter.

#### RESULTS

*Equilibration Time.* The length of infusion necessary to insure adequate and uniform distribution of inulin throughout the extracellular space has been determined experimentally. We consider that equilibrium is reached when any further prolongation of the infusion will not increase the inulin volume.

Determinations were repeatedly performed in the same animal with varying durations of infusion. Twenty-one experiments in 5 normal dogs show that equilibrium is obtained within 2 hours (table 1, *experiments 1-21*). In dog 1 the infusion was maintained for 2, 3.3, 4.5, and 12 hours respectively with no significant change in the volume of distribution.

Three normal dogs were nephrectomized bilaterally and given a single injection of inulin. The plasma concentration was determined every 20 to 30 minutes during

the first 2 hours, and every hour thereafter. The observations were prolonged for 12 hours. The time necessary for uniform distribution varied from 1 to 2 hours (fig. 1), confirming the observations made in the normal dogs. Thiocyanate, injected simultaneously, reached a constant plasma concentration in 40 to 90 minutes.

As the inulin and thiocyanate spaces were followed, it became apparent that after equilibrium had been established and maintained for about 6 hours, both spaces began to increase (fig. 1). It is probable that this represents a terminal pathological shift of intracellular water in the nephrectomized animal. The results obtained in

TABLE I. VOLUMES OF DISTRIBUTION OF INULIN, THIOCYANATE AND RADIOACTIVE SODIUM IN DOGS

EXPER.	DOG	BODY WT. kg.	INULIN					VOLUME OF DISTRIBUTION (% BODY WT.)		
			Duration of infusion hr.	Inulin recovered mg.	Urine flow cc/min.	Delay time correction	Inulin space l.	Inulin	Thio- cyanate	Sodium <sup>24</sup>
								% amt. recovered	l.	
1	1	17.0	2.0	662	.5	16.4	3.240	19.0	35.5	
2	1	16.4	3.3	635	.8	14.2	3.260	19.8	34.3	31.4
3	1	19.0	4.5	1011	3.5	2.8	3.500	18.7	27.0	
4	1	19.0	12.0	967	3.6	2.8	3.540	18.7	34.5	27.5
5	2	18.0	2.0	362	1.4	6.9	3.510	19.5	32.0	31.0
6	2	17.1	2.5	251	3.8	4.0	3.000	17.6		32.4
7	2	17.0	3.3	231	2.2	7.0	3.120	18.4	29.9	
8	2	19.3	3.5	784	2.2	4.7	3.830	19.8		
9	2	16.8	4.0	255	1.3	6.7	2.940	17.5	34.4	
10	3	15.0	3.3	202	3.7	5.0	3.040	20.3	32.5	26.7
11	3	15.0	3.8	167	2.2	6.0	3.020	20.1	30.7	
12	3	15.8	4.0	225	1.6	8.0	3.180	20.1	28.5	
13	4	13.5	3.3	182	1.0	11.0	2.580	19.1	25.8	33.0
14	4	13.8	5.8	103	1.3	8.3	2.730	19.8		
15	5	13.3	2.3	230	3.0	3.5	2.640	19.9	31.6	30.5
16	5	13.0	2.4	250	4.0	3.2	2.290	17.6		
17	5	12.5	2.8	193	2.3	6.7	2.370	19.0	31.2	30.5
18	5	12.8	3.0	329	4.6	3.7	2.330	18.2	35.6	
19	5	12.5	3.8	303	.6	13.2	2.550	20.4	30.8	
20	5	12.5	4.3	340	.9	10.3	2.600	20.8	37.5	
21	5	11.5	4.5	280	.7	15.0	2.470	21.4	40.0	
22	6	10.8	3.0	314	1.5	7.7	2.350	21.8	33.7	33.7
23	6	10.1	3.1	303	1.9	5.0	1.910	18.9	30.2	
24	6	10.5	3.6	326	1.3	7.4	2.050	19.6	33.9	31.6
25	7	17.5	2.1	380	3.0	4.0	3.380	19.3	38.0	
26	8	14.5	3.0	208	.6	19.0	2.870	19.8	35.0	

the normal animal (table I, dog 1) in which the inulin infusion was maintained for 12 hours with no appreciable increase in the inulin space supports this view.

**Delay Time.** The total inulin excreted after the infusion is discontinued constitutes not only the inulin in the extracellular space but also a certain amount contained within that portion of the urinary tract between Bowman's capsule and the bladder (dead space) at the instant of the cessation of the infusion. For the accurate measurement of the inulin space, the inulin in the dead space must be subtracted from the total recovery. This correction has been estimated in the following way.

During the infusion, control urine collections were made at intervals of 2 minutes. The infusion was then stopped at the end of one such interval, and similar col-

lections were continued for the next 10 or 15 minutes. If the inulin clearance is constant, the amount excreted per minute during the infusion is likewise constant.

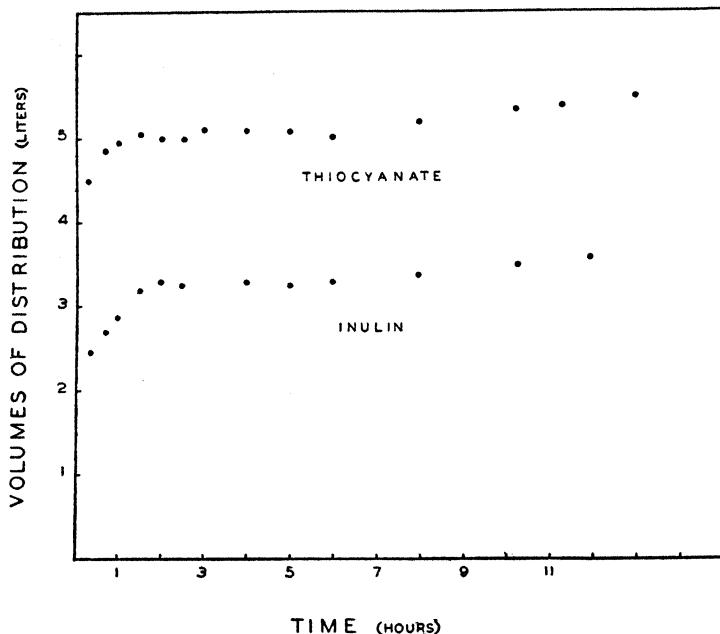


FIG. 1. EQUILIBRATION of inulin and thiocyanate in a nephrectomized dog. The injections were given at time zero. The dots represent calculated spaces of distribution from determined plasma levels. Two other dogs showed similar curves.

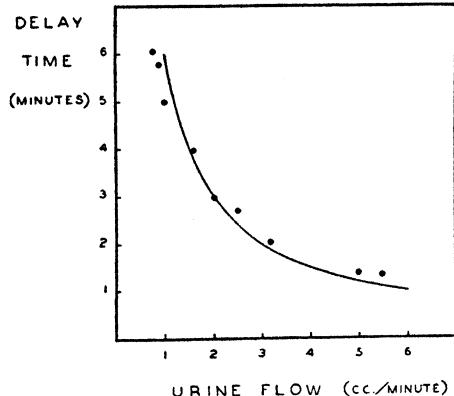


FIG. 2. RELATION between delay time and urine flow. The dots represent the results of 9 experiments performed in 4 normal dogs of similar size.

Experimentally it was determined that the plasma level falls immediately after the infusion is discontinued. The time elapsing between the fall in plasma level and the subsequent fall in the rate of excretion is the delay time. This time in minutes multi-

plied by the rate of excretion in mg. per minute equals the mg. of inulin in the dead space. The delay time was thus measured in 4 dogs with urine flows varying from 0.8 to 5.5 cc. per minute (fig. 2). It is clear that the delay time is inversely proportional to the urine flow, and that their product is therefore constant. This constant, equivalent to about 6 cc. in the dog, is a measure of the dead space.

The delay time is negligible when the urine flow is over 5 cc. per minute, it adds a 5 per cent error to the inulin space when the urine flow is 2.5 cc. per minute, and causes a larger error with urine flows less than 1 cc. per minute. All the inulin spaces reported in this paper have been corrected for appropriate delay times. The average correction in 26 experiments with varying urine flows amounts to 8 per cent of the uncorrected value (table 1).

*Normal Values of the Inulin Space.* The volume of distribution of inulin as determined by the above method was measured in 26 experiments performed in 8 normal dogs (table 1). In all instances, the infusion was maintained for at least 2 hours to insure complete and uniform distribution. The average corrected inulin space is 19.4 per cent of the body weight, with a range from 17.5 to 21.8 per cent.

TABLE 2. VOLUMES OF DISTRIBUTION OF INULIN, THIOCYANATE AND SODIUM<sup>24</sup>  
BEFORE AND AFTER TOTAL NEPHRECTOMY

Dog	Body wt. kg.	CONTROL SPACES						SPACES AFTER NEPHRECTOMY						
		Inulin		Sodium <sup>24</sup>		Thiocyanate		Inulin		Sodium <sup>24</sup>		Thiocyanate		
		l.	% body wt.	l.	% body wt.	l.	% body wt.	l.	% body wt.	l.	% body wt.	l.	% body wt.	
A	15.0	3.900	26.0	4.650	31.0	5.640	37.6	13.3	34.60	26.0	4.590	34.6	5.700	43.0
B	16.5	3.380	20.4	6.500	30.4	10.0	31.30	20.6	4.040	25.3	5.000	31.3		

The average thiocyanate space in these animals is 33.8 per cent of the body weight with a range of 25.8 to 40 per cent. The Na<sup>24</sup> space averages 30.4 per cent with a somewhat smaller scatter than seen in the thiocyanate values.

*Nephrectomized Dogs.* To corroborate the spaces obtained with the infusion method, control measurements of inulin, thiocyanate, and Na<sup>24</sup> spaces were made in normal anesthetized dogs, and then repeated after complete nephrectomy (table 2). The control inulin spaces were determined with the infusion method and the post-nephrectomy spaces with the single injection technique. The inulin space before and after nephrectomy proves to be the same (table 2).

#### DISCUSSION

The method here described assures the uniform distribution of inulin throughout the extracellular fluid, despite its rapid excretion.

The delay time correction has eliminated a source of error which was not considered in the preliminary paper (30). The average inulin space so corrected proves to be 19.4 per cent instead of the previously reported value of 21.6 per cent of the body weight.

The rate of excretion of the urinary inulinoid blank varies throughout the day.

This fact may introduce an error which can be avoided by elevating the plasma level 3- to 4-fold<sup>8</sup>, when the blank correction is reduced to negligible proportions.

The observations in control and nephrectomized animals confirm the inference that the infusion method measures the same space in the normal animal that the single injection method measures in the nephrectomized dog.

The arguments which favor the conclusion that inulin does not enter the cells have been mentioned above. Further, Wilde (13) has shown that in nephrectomized rats both inulin and sucrose, substances of vastly different molecular weights, have identical volumes of distribution.

Previous methods of measuring extracellular fluid have employed substances which are now known to enter cells or to be partially metabolized. The circumstance that the inulin space affords the lowest value of extracellular fluid yet recorded argues that it represents the best described approximation of that value. The only other alternative would be that inulin has diffused incompletely into the extracellular compartment. In table 1 it is demonstrated that if the infusion is maintained as long as 12 hours, a period approximately 6 times the required equilibration time, there is no further increase in inulin space. It is improbable that if after 2 hours diffusion is incomplete, it will remain so after 12 hours. In view of our results, we believe that inulin is distributed, at equilibrium, throughout the extracellular fluid volume.

Using deuterium oxide (33, 34), the total body water in 6 normal dogs has been found to average 63 per cent of the body weight, range 55.7 to 74.3 (35). As calculated by the difference between the total body water and the measurement of the extracellular fluid here reported, the intracellular water content averages 44 per cent, range 36.3 to 54.9.

From table 1 it is apparent that the ratio of the 3-hour  $\text{Na}^{24}$  space to the inulin space is approximately 3/2. Consequently we conclude that about one third of the total sodium in the dog is intracellular.

#### SUMMARY

A new method has been devised which permits the use of inulin as a measure of the extracellular space, by means of an equilibrating infusion followed by the collection of the total inulin excreted, after the cessation of the infusion. The length of infusion necessary to insure uniform distribution of inulin throughout the extracellular space is 2 hours in the dog. A method is described to measure the delay time which permits the correction of the total inulin recovery. This delay time is inversely proportional to the urine flow. The product of delay time and urine flow is constant and a measure of the dead space. The dead space in the dogs used is 6 cc. The average extracellular space of the dog is 19.4 per cent of the body weight compared to 30.4 per cent and 33.8 per cent for the spaces of  $\text{Na}^{24}$  and thiocyanate respectively.

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<sup>8</sup> A concentration of 30 to 50 mg. per cent does not exert any significant osmotic effect.

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# RATE OF ENTRANCE OF URATE AND ALLANTOIN INTO THE CEREBROSPINAL FLUID OF THE DALMATIAN AND NON-DALMATIAN DOG<sup>1</sup>

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**O**BSERVERS (1-3) have reported that the concentration of urate in cerebrospinal fluid (C.S.F.) is only a small fraction of that found in plasma or serum. The reason for this difference in concentration is unknown largely because of lack of precise information concerning (1) formation of the C.S.F. and (2) the physicochemical state of urate in the plasma. Although the weight of opinion (4-6) is that the blood-cerebrospinal fluid barrier possesses peculiarly selective properties, Wolfson *et al.* (3) recently postulated that the C.S.F. was essentially a dialysate or ultrafiltrate of plasma and that the small concentration of urate in it resulted primarily because of the relatively non-diffusible state of urate in plasma. This latter group, moreover, interpreted (7) the low rate of renal clearance of urate occurring in most mammals as resulting from inability of urate to pass the glomerular membrane, rather than from its partial renal tubular reabsorption.

Urate has been shown by us (8) to be diffusible through the glomeruli of the Dalmatian dog. In this animal, therefore, a direct determination may be made as to whether diffusibility of urate itself is of primary importance with regard to its entrance into the cerebrospinal fluid.

The concentration of allantoin in cerebrospinal fluid has not hitherto been determined. This substance is the principal excretory end product of purine metabolism in sub-primate mammals (except for the Dalmatian dog, where it shares this role with uric acid), and is therefore the physiological analog of uric acid in these animals. We have shown (9, 10) that allantoin is freely diffusible through the glomeruli of the rat, dog, and man. It was therefore of interest to us to determine the extent to which this freely diffusible substance is present in the C.S.F., and the rate at which it enters that fluid.

The results of our investigation indicate that some selective hindrance exists to the passage of both urate and allantoin from the plasma to cerebrospinal fluid of Dalmatian dogs, and a similar hindrance exists to the passage of allantoin and of injected urate from the plasma to the C.S.F. of non-Dalmatian dogs.

## METHODS AND RESULTS

*Concentration of Urate and Allantoin in the Plasma and C.S.F. of the Non-Dalmatian and Dalmatian Dog.* Nineteen non-Dalmatian and 2 Dalmatian dogs were

Received for publication January 3, 1949.

<sup>1</sup> Aided by grants from The Public Health Service and the Wine Advisory Board of the State of California.

studied. Samples of C.S.F. (2 cc.) were obtained by the intraspinal insertion of a lumbar spinal needle (20 gauge) between the second and third cervical vertebrae of dogs anesthetized with sodium pentobarbital. Immediately after each sample of C.S.F. was obtained, a sample of blood was obtained, by puncture of the femoral artery.

Twenty-one pairs of samples of plasma and C.S.F. obtained from 18 non-Dalmatian dogs and 8 similar samples of plasma and C.S.F. from 2 thoroughbred Dalmatian dogs were analyzed for their urate content. Ten plasma and 11 C.S.F. samples obtained from 10 non-Dalmatian dogs and 5 similar samples of plasma and C.S.F. obtained from 2 Dalmatian dogs were analyzed for their allantoin content. Urate, allantoin and creatinine determinations were done according to previously described methods (8, 9).

The average plasma urate concentration of non-Dalmatian dogs was found to be 0.23 mg/100 cc. (S.E. mean  $\pm$  0.016), with a range from 0.10 to 0.45 mg/100 cc. The average concentration of urate in the C.S.F. of these dogs was 0.24 mg/100 cc. (S.E. mean =  $\pm$  0.014) with a range of 0.13 to 0.35 mg/100 cc. Although the average concentration and range of urate in the C.S.F. of normal dogs was approximately the same as that in their plasma, no correlation was established between the individual paired samples of plasma and C.S.F. Perhaps the necessary chemical error involved in the analyses of such minute samples led to this seeming lack of correlation.

The average plasma urate concentration of 2 Dalmatian dogs was 0.48 mg/100 cc. (S.E. mean =  $\pm$  0.038) and ranged between 0.30 and 0.65 mg/100 cc. for the 8 samples. The average concentration of urate in the C.S.F. of Dalmatians was 0.24 mg/100 cc. (S.E. mean =  $\pm$  0.027) and ranged from 0.18 to 0.30 mg/100 cc. The average concentration of urate in Dalmatian C.S.F. was identical with that in normal dogs, despite the fact that Dalmatian plasma urate concentration was twice that of normal dogs. The ratio of urate in plasma to urate in spinal fluid of Dalmatians varied from 1.6 to 3.0.

The average plasma allantoin concentration of non-Dalmatian dogs was 1.47 mg/100 cc. (range: 1.10 to 1.85 mg/100 cc.). The allantoin content of their C.S.F., however, was only 0.30 mg/100 cc. (range: 0.25 to 0.47 mg/100 cc.) or 80 per cent less than that of plasma. In the Dalmatian dogs, the average plasma allantoin concentration (0.68 mg/100 c.) was below that of the non-Dalmatian dogs but here also the average allantoin concentration of their C.S.F. (0.14 mg/100 cc.) was approximately 80 per cent below the plasma level.

In other words, there was a difference between the C.S.F. and plasma concentration of both urate and allantoin of normal Dalmatian dogs, and a similar difference in the allantoin levels of normal non-Dalmatian dogs; this difference is maintained despite the fact that plasma urate in the Dalmatian and plasma allantoin in all dogs are in diffusible form.

*Rate of Entrance of Allantoin into the C.S.F. of Non-Dalmatian Dogs Following Elevation of the Plasma Allantoin Content.* Although the above results indicated that under normal conditions allantoin was not distributed equally between plasma and C.S.F. of both types of dogs, it was thought desirable to study the change in concentration of allantoin in the C.S.F. after elevation of the plasma allantoin content.

Five non-Dalmatian dogs were anesthetized with sodium pentobarbital. Control blood and C.S.F. samples were taken, then a solution containing 500 mgm. of allantoin per 100 cc. of normal saline solution was infused intravenously at the rate of 3 cc. per minute. Blood samples were taken 30 minutes after the infusion had been started and again at the end of 90 minutes, at which time the second C.S.F. samples also were obtained. All samples were analyzed for their allantoin content.

As table 1 demonstrates, although the average plasma allantoin concentration in the 5 dogs during the perfusion period was 8.90 mg./100 cc., the average allantoin content of the C.S.F. at the end of the 90-minute period was only 0.68 mg./100 cc. as compared to the average initial concentration of 0.31 mg./100 cc. When compared to the gain in the creatinine concentration of C.S.F. resulting from a similar elevation of plasma creatinine in other dogs, it can be seen (compare tables 1 and 3) that approximately twice as much plasma creatinine had entered the C.S.F. in the same period of

TABLE 1. CHANGE IN ALLANTOIN CONTENT OF C.S.F. OF DOGS AFTER ELEVATION OF PLASMA ALLANTOIN BY INTRAVENOUS INFUSION

DOG	PLASMA CONC. OF ALLANTOIN BEFORE AND DURING INFUSION mgm./100 cc.				C.S.F. CONC. OF ALLANTOIN BEFORE AND DURING INFUSION mgm./100 cc.	
	Bef.	30 Min.	90 Min.	Av. <sup>1</sup>	Bef.	90 Min.
N1.....	1.5	14.2	17.1	10.9	0.31	0.69
N2.....	1.1	11.6	16.8	9.8	0.23	0.59
N3.....	1.5	10.9	13.5	8.6	0.25	0.75
N4.....	1.8	8.9	12.1	7.6	0.47	0.70
N5.....	1.5	11.1	10.0	7.5	0.30	0.69
Av.....	1.5	11.3	13.9	8.9	0.31	0.68

<sup>1</sup> Equals average concentration of allantoin in plasma during entire infusion (average of the preceding 3 determinations).

time. These observations suggested the presence of a selective barrier to the entrance of allantoin into the C.S.F.

This relative impermeability of the blood brain barrier to diffusible plasma allantoin was also shown in a second experiment in which 2 normal dogs were nephrectomized. As table 2 demonstrates, the average allantoin content of C.S.F. (3.81 mg./100 cc.) in the 2 dogs 72 hours after nephrectomy was still much less than that found in plasma only 24 hours after nephrectomy. Likewise the creatinine content of C.S.F. (2.11 mg./100 cc.) of the 2 dogs 72 hours after nephrectomy was less than half of that present in plasma only 24 hours after nephrectomy. However, the ratio of plasma creatinine to C.S.F. creatinine before and during the 72 hours following nephrectomy was always much less than the ratio of plasma allantoin to C.S.F. allantoin. These studies indicated that there was a failure to achieve equality between the concentration of allantoin in plasma and C.S.F. and a similar although less marked failure in the case of creatinine.

*Rate of Entrance of Urate into the C.S.F. of Non-Dalmatian and Dalmatian Dogs Following the Elevation of Plasma Urate Content.* Five non-Dalmatian dogs were anes-

thetized and after control blood and C.S.F. samples were obtained, they were given an intravenous infusion of a solution containing 500 mg. of urate, 200 mg. of creatinine, and 250 mg. of lithium carbonate per 100 cc. This infusion was given at the rate of 7 cc. per minute and maintained for 90 minutes. Blood samples were taken at 30 and again at 90 minutes, at which latter time a second C.S.F. sample was obtained. All samples were analyzed for their urate and creatinine contents. The same procedure was performed 4 times on 2 Dalmatian dogs.

Despite the lower average concentration of creatinine maintained during the period of infusion (table 3) approximately twice as much creatinine (0.67 mg/100 cc.)

TABLE 2. CHANGE IN ALLANTOIN AND CREATININE CONTENT OF PLASMA AND C.S.F. AFTER BILATERAL NEPHRECTOMY

TIME AFTER NEPHRECTOMY	DOG	PLASMA		C.S.F.		RATIO (PLASMA/C.S.F.)	
		ALLANT.	CREAT.	ALLANT.	CREAT.	ALLANT.	CREAT.
<i>Hours</i>		<i>Mg/100 cc.</i>		<i>Mg/100 cc.</i>			
Control	N6	1.57	0.58	0.20	0.52	6.05	1.13
	N7	1.94	0.76	0.44	0.50	4.44	1.52
Average.....		1.76	0.67	0.35	0.51	5.25	1.32
24	N6	11.8	3.90	0.98	1.10	12.1	3.55
	N7	14.2	4.70	0.99	1.11	9.0	4.25
Average.....		13.0	4.30	0.99	1.11	10.6	3.90
48	N6	18.7	6.79	2.17	1.49	9.0	4.60
	N7	23.0	7.29	2.96	1.62	7.4	4.60
Average.....		20.85	7.04	2.57	1.56	8.2	4.6
72	N6	25.3	9.20	3.54	2.23	7.2	4.1
	N7	27.6	9.30	4.07	1.98	6.8	4.7
Average.....		26.45	9.25	3.81	2.11	7.0	4.4

entered into the C.S.F. of non-Dalmatian dogs as did urate (0.39 mg/100 cc.). The same relative disproportion was observed (table 3) in the Dalmatian dogs. There seemed little question then that even the freely diffusible plasma urate of the Dalmatian dog was differentially hindered in its entrance into the C.S.F. Moreover, when the rate of urate transfer from plasma to C.S.F. of the non-Dalmatian dog was compared with that of Dalmatian, the two were observed (table 3) to be similar, suggesting the physicochemical similarity of the plasma urates in each type of dog.

*Rate of Disappearance of Injected Urate from the C.S.F. of Non-Dalmatian Dogs.* Five male dogs were anesthetized and immediately after blood and C.S.F. samples were obtained, 1 cc. of a solution containing 500 mg. of lithium urate and 500 mg. of creatinine per 100 cc. was injected into the cerebrospinal canal. Equal distribution of this solution throughout the C.S.F. was attempted by rapid withdrawal and re-

TABLE 3. CHANGE IN URATE AND CREATININE CONTENT OF C.S.F. OF DOGS AFTER ELEVATION OF PLASMA URATE AND CREATININE BY INTRAVENOUS INFUSION

DOG	AVERAGE PLASMA CONCENTRATION DURING INFUSION <sup>1</sup>		AVERAGE C.S.F. CONCENTRATION BEFORE AND AT END OF INFUSION				GAIN IN C.S.F. URATE AND CREATININE AT END OF INFUSION		
	Urate	Creat.	Bef. Inf.		End Inf. (2)		Urate	Creat.	
			Urate	Creat.	Urate	Creat.			
<i>Non-Dalmatian Dogs</i>									
N8.....	10.9	10.1	0.25	0.60	0.83	1.42	0.58	0.82	
N9.....	7.7	7.7	0.29	0.64	0.59	1.24	0.30	0.60	
N10.....	6.8	7.7	0.27	0.64	0.76	1.31	0.49	0.67	
N11.....	11.2	5.3	0.19	—	0.41	—	0.22	—	
N12.....	11.8	4.6	0.24	0.69	0.62	1.26	0.38	0.57	
Av.....	9.68	7.08	0.25	0.64	0.64	1.31	0.39	0.67	
<i>Dalmatian Dogs</i>									
LD.....	8.4	8.1	0.18	0.49	0.62	1.55	0.44	1.06	
LD.....	8.2	7.5	0.22	0.48	0.42	1.29	0.20	0.81	
LD.....	12.0	5.8	0.30	0.58	0.77	1.13	0.47	0.55	
SD.....	8.8	8.3	0.24	0.52	0.53	1.35	0.29	0.83	
Av.....	9.4	7.4	0.24	0.52	0.59	1.33	0.35	0.81	

<sup>1</sup> Equals average concentration of urate and creatinine in plasma during entire infusion (average of initial concentration, that after 30 minutes and that after 90 minutes of infusion).

<sup>2</sup> Equals concentration of urate and creatinine in C.S.F., 90 minutes after infusion had been begun.

TABLE 4. RATE OF DISAPPEARANCE OF INJECTED URATE AND CREATININE FROM C.S.F. OF DOGS

DOG	BEFORE INJECTION		AFTER INJECTION							
			30 Min.		60 Min.		120 Min.		24 Hours	
	Urate	Creat.	Urate	Creat.	Urate	Creat.	Urate	Creat.	Urate	Creat.
	Mg/100 cc.		Mg/100 cc.		Mg/100 cc.		Mg/100 cc.		Mg/100 cc.	
N13.....	0.20	0.49	53.4	55.2	37.1	40.6	22.9	23.9	—	—
N14.....	0.30	0.58	38.0	43.0	26.0	35.0	19.6	21.0	—	—
N15.....	0.20	0.44	19.6	21.0	13.0	16.0	7.4	8.0	—	—
N16.....	0.34	0.71	42.0	48.0	30.0	37.0	18.0	18.0	0.58	0.85
N17.....	0.28	0.60	31.0	37.0	19.0	19.0	11.0	9.2	0.38	0.73
Av.....	0.26	0.56	36.8	40.8	25.0	29.5	15.8	16.0	0.48	0.79

injection of 5 cc. of the C.S.F. 7 times by means of a syringe. Samples of C.S.F. were obtained 30, 60, and 120 minutes after this injection. Samples were obtained in 2 dogs 24 hours after injection. Blood samples also were obtained at 2 and at 24 hours

after injection and were analyzed for urate, allantoin, and creatinine. The samples of C.S.F. were analyzed for their urate and creatinine content.

Unlike the discrepancy observed between the rates of penetration of plasma urate and creatinine into the C.S.F., the rate of disappearance of these substances from the C.S.F. after injection therein was approximately equal (table 4). It is of interest that even after 24 hours not all of the excess urate or creatinine had disappeared from the C.S.F. However, the relatively small amount of either substance entering the blood from the C.S.F. could not be detected, as determinations of urate, allantoin, and creatinine in the plasma obtained 2 and 24 hours after injection from the dogs showed no essential change in the concentration of any of the 3 substances.

#### DISCUSSION

Recently Wolfson *et al.* (3) (assuming that C.S.F. was a modified dialysate or ultrafiltrate) conjectured that plasma urate was in a relatively non-diffusible state because of the relative paucity of urate in human C.S.F. On the basis of this latter conjecture, moreover, they were led to believe (7) that only a small fraction of total plasma urate was filtered through the glomerulus.

Our observations, however, were not in agreement with the above views. It appeared that the urate content of the C.S.F. was not strictly dependent upon dialysis or ultrafiltration of diffusible plasma urate but more perhaps upon the selective functions of the blood brain barrier. The content of urate in the C.S.F. of the ordinary dog and in the Dalmatian was the same although the plasma urate of the latter was twice that of the former and in a freely diffusible state (8). Moreover, when urate was given intravenously to these dogs, a similar degree of selective hindrance toward entrance of urate (as compared with the entrance of creatinine) into the C.S.F. was found in each type of animal, although the increased urate in the plasma of the Dalmatian was known to be in a diffusible state (8).

Similarly, despite the known diffusibility of allantoin in the plasma of both types of dogs (9), a marked discrepancy existed between the allantoin content of plasma and C.S.F. either at normal or at increased levels of plasma allantoin. This suggested again that the C.S.F. (in respect to allantoin also) was neither dialysate nor ultrafiltrate.

Our data therefore suggest that the low concentration of urate in C.S.F. of man is due not to the assumed non-diffusibility of plasma urate but to the demonstrated selective discrimination exercised by the blood brain barrier (4-6).

Moreover, considerable direct evidence is available that plasma urate in man and other animals is in a diffusible, hence filtrable state. Thus the concentration of urate in lymph (11), pleural fluid (12, 13), joint fluid (14, 15) and ascitic and pericardial fluid (12, 13) has been found to equal that of plasma. More importantly, Bordley and Richards (16) have demonstrated that the glomerular urine of the snake and frog contains as much urate as their plasma.

In view of these observations, it would seem hazardous to consider plasma urate either non-diffusible or non-filtrable through the glomerulus because of its low concentration in a fluid (C.S.F.) which has not been found to be either a true dialysate or ultrafiltrate (4-6).

## SUMMARY

The allantoin and urate concentration in the cerebrospinal fluid of both non-Dalmatian and Dalmatian dogs was determined. The blood brain barrier of both types of dogs was found to be selectively discriminatory against the entrance of allantoin. However, the concentration of urate in the C.S.F. and plasma of non-Dalmatian dogs was the same at normal plasma levels, but when the plasma urate was artificially elevated, hindrance to the entrance of plasma urate into C.S.F. was observed and to the same extent as that found in the Dalmatian dogs. The state of diffusibility and glomerular filtrability of plasma urate in non-Dalmatian and Dalmatian dogs was discussed.

The authors express their thanks to Ann Farrell and Catherine Bland for technical assistance in the execution of this study.

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# REDUCTION OF PLASMA POTASSIUM CONCENTRATION OF THE DOG BY VIVODIALYSIS AND ITS RES- TORATION IN NON-VISCERAL REGIONS

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**I**T HAS been found possible to remove sufficient potassium by vivodialysis within a few hours to indicate that potassium can move from other regions to replace that withdrawn from the extracellular fluid (1). Since the non-visceral regions, composed chiefly of skeletal muscle, skin, bone and associated connective tissue, contain the greater part of the body's potassium (2), it is of interest to determine whether or not they can supply potassium to sustain the concentration in the extra cellular fluid. To obtain evidence bearing on this question a study was made on the plasma potassium concentration in the arterial and femoral vein blood during the removal of potassium by vivodialysis.

The methods were essentially those reported previously (1). Small dogs were used in order to increase the relative capacity of the dialyzing equipment. This necessitated filling the dialyzer with heparinized blood from donor dogs before beginning the dialysis. It was also necessary to give small transfusions from time to time to compensate for sampling and incidental blood loss. These were given into the line carrying blood to the dialyzer so that the potassium was removed before it reached the dog. Blood for dialysis was drawn from the vena cava by a catheter passed through a femoral vein and returned through a cannula tied into a tributary of an external jugular vein. Arterial samples were taken from a 1 mm. O.D. polyethylene catheter (obtainable from Anchor Plastics Co., New York City) in the ipsilateral femoral artery and femoral vein samples from a similar catheter passed into the contralateral femoral vein in the distal direction. These catheters were passed into the respective vessels through needles introduced obliquely through the exposed vessel wall. When the needles were stripped off there was no serious bleeding. The catheters did not seem to interfere with blood flow. In two animals the intestinal mesentery was exposed by a short midline incision into the belly, and a slender polyethylene catheter was passed through one of the intestinal veins into the portal vein. The essential circumstances of each experiment such as the size of the animal and the extent of the dialysis are shown in table 1.

## RESULTS AND DISCUSSION

More extensive removals of potassium relative to the size of the animal were achieved than in the earlier study (1) (table 1). These potassium removals effected substantial reductions in the arterial plasma potassium level (fig. 1). In some instances these ranged below the values reported in hypopotassemia due to potassium-

Received for publication March 7, 1949.

deficient diets (3) or the treatment of diabetic coma with insulin (4). Only in patients suffering from periodic familial paralysis and given insulin or glucose have

TABLE I

EXPER.	WEIGHT	PUMP RATE		VOLUME OF DIALYZING FLUID USED	ELAPSED TIME OF DIALYSIS	EXTRA BLOOD GIVEN		TOTAL K IN DIALYZING FLUID	K REMOVED FROM DOG	K IN EXTRACELLULAR FLUID INITIALLY
		Blood	Dialyzing fluid			Volume	Plasma K content			
	kg.	cc./min.		l.	min.	cc.	mg.	mg.	mg.	mg.
I ♂	10.9	93-98	239	51.9	219	350	30	723	693	513
II ♀	7.2									
III ♂	8.8	97-103	267	69.1	262	500	53	1099	1046	470
IV ♀	10.3	86-97	239	88.5	461	400	41	1523	1482	515
V ♀	10.0	54-102	232	94.0	429	880	110	1656	1546	429
VI ♀	10.0	90-101	219	87.5	399	600	75	1482	1407	459

Elapsed time of dialysis includes the time during which dialysis was interrupted. The potassium in extracellular fluid initially was calculated by multiplying the initial concentration in the plasma by 30% of the animal's weight. The potassium removed from the dog equals the total amount in the dialyzing fluid minus that in the plasma of the extra blood given. All of the animals were allowed to fast for about 2 days prior to dialysis.

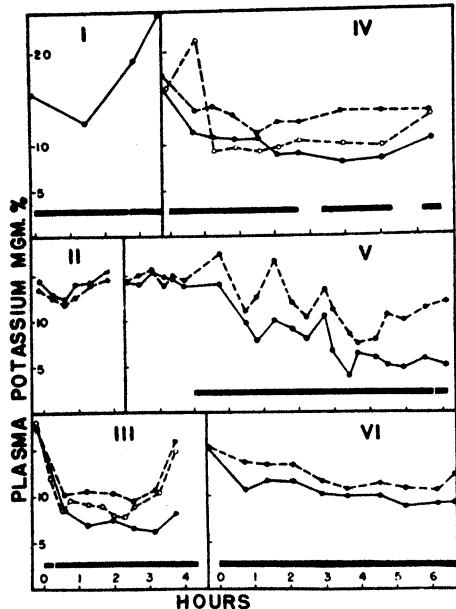


Fig. 1. DOGS WERE UNDER SODIUM PENTOBARBITAL ANESTHESIA and heparinized in all instances. In experiment I, the blood was both drawn and returned to the posterior vena cava. Although the return was proximal, yet the pump rate for blood was sufficiently great to cause retrograde flow in the vena cava and sufficient recirculation to reduce the efficiency of dialysis in the smaller size of dog. The last samples taken in all experiments were agonal. Arterial: solid lines, solid circles; femoral vein: broken lines, solid circles; portal vein: broken lines, open circles. Horizontal bars indicate the periods during which the dialyzer was operating.

equally low values been found (5). The evaluation of this finding, however, would necessitate a comparative study of the methods used in determining potassium. The predialysis values, however, were within the range found by others (6) for dogs under sodium pentobarbital anesthesia.

The femoral vein plasma potassium was sustained at a level distinctly higher than the arterial during the period when this was reduced by potassium removal. The difference was so large and maintained over such prolonged periods that blood flows so small as to be unlikely must be assumed in order to explain it on any basis other than the movement of potassium into the extracellular fluid within the region. Skeletal muscle, skin, bone and erythrocytes may be considered as possible sources of this potassium. Since both the total amount and the concentration of potassium in the erythrocytes in the body of the dog are small (7), they seem to be unlikely as a source. Conversely because of both the high concentration and the large total amount within skeletal muscle, this tissue must be considered first among likely sources of potassium (2). The result reported here is in accord with earlier studies in which it was found that the hind limbs of the frog would give up potassium to a perfusing fluid poor in potassium (8).

Several factors have been found to affect the plasma potassium concentration (9). Some one or combination of these probably was responsible for the fluctuations appearing in the femoral vein concentrations. An agonal rise, probably on the same basis, was apparent in most of the experiments.

The smaller arterio-venous increases found in the portal vein samples may indicate relatively as great a mobilization of potassium from the viscera drained as from the hind limb if the difference in the rate of blood flow is taken into account (10).

#### SUMMARY

The arterial plasma potassium level of the dog was substantially reduced by vivodialysis. Concurrently there was a definite arterio-venous increase in the plasma potassium concentration of blood flowing through the hind limb.

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# CREATINURIA FROM GUANIDOACETIC ACID INGESTION AND ITS RELATION TO THE SITE OF ACTION OF METHYLTESTOSTERONE<sup>1</sup>

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**I**N NO experiments in which creatine has been administered to man or other animals has the administered creatine been recovered quantitatively either as creatine or creatinine (1, 2). Three explanations for the apparent loss of administered creatine have been offered: 1) that it may be converted to some compound other than creatine, 2) that it may be partly destroyed in the intestine by bacteria, and 3) that it may retard the synthesis of creatine in the body. The first hypothesis is discredited by the experiments of Bloch and Schoenheimer (3) and du Vigneaud (4) with isotopic creatine. The second hypothesis, emphasized by Bodansky (5), has been tested only under limited conditions. Recently in this laboratory a subject, whose tissue-creatine had been labeled with N<sup>15</sup>, ingested large quantities of non-isotopic creatine (6). The ingested creatine could not be accounted for in either the tissue depots or the excreta. It appeared that during and for approximately 10 days after the ingestion of creatine the synthesis of endogenous creatine was greatly retarded.

In keeping with this inference the excretion of guanidoacetic acid, the precursor of creatine, increased. To account for the total deficiency in the production of creatine it was suggested that guanidoacetic acid is not entirely converted to creatine. Studies of the effect of methyltestosterone, however, indicated that this compound accelerates the synthesis of guanidoacetic acid, which in turn is methylated to creatine (7, 8). Creatinuria appears when the capacity of the tissues to store creatine is exceeded. The results of the two sets of experiments seemed to be contradictory. If the presence of an excess of creatine inhibits the methylation of guanidoacetic acid, the synthesis of endogenous creatine should be reduced to minimum when the tissue depots are saturated with creatine. To resolve the conflict it is necessary to postulate either that methyltestosterone accelerates the methylation as well as the synthesis of guanidoacetic acid or that there is an alternate route for the disposition of excess creatine in the body.

In the present experiments tolerance to guanidoacetic acid was studied before and after ingestion of creatine to determine whether its methylation was in fact retarded by excess creatine. The conversion of guanidoacetic acid to creatine was also studied after the tissues had been loaded to capacity with creatine. Under these conditions there was no significant evidence that the conversion of guanidoacetic acid

Received for publication February 23, 1949.

<sup>1</sup> Aided by a grant from the Fluid Research Fund of Yale University School of Medicine.

to creatine was retarded. In fact, it has been possible to simulate the creatinuria of methyltestosterone simply by administering guanidoacetic acid. In addition it has been found that in man an increase in reabsorption of creatine by the tubules of the kidneys is associated with a reduction in reabsorption of guanidoacetic acid (9). This provides an alternate explanation for the increased excretion of guanidoacetic acid during administration of creatine.

#### PROCEDURE

The author served as subject and, except during periods in which large quantities of creatine were ingested, received a diet free from creatine, which contained an average of 90 gm. of protein and calories adequate to maintain weight. This was supplemented with 250 cc. of milk per day. To simulate physiological conditions as closely as possible when creatine or guanidoacetic acid (GAA) was ingested over the periods indicated in the figures, the quantities were divided into 16 portions which were taken in capsules at hourly intervals. In the case of daily quantities greater than 2.5 gm. 32 portions were taken at half-hour intervals over the same period. Twenty-four-hour urine collections were terminated with the over-night specimen approximately at the same hour each day, and all values were corrected to the quantity excreted per 24 hours. When GAA tolerance tests were made, 1.5 gm. were ingested in 5 equal hourly doses at the beginning of the collection period. Single blocks in the figures extending over several days represent instances in which daily urine collections were pooled by taking aliquots proportional to their volumes in order to prevent summation of analytical errors. When single daily urines were analyzed there was considerable fluctuation in the values for creatine. Since each low value was followed by a correspondingly high value and the averages showed a high degree of consistency, it is assumed that the fluctuations were due to incomplete voiding of the concentrated overnight urine.

#### METHODS

The analytical methods for GAA and for creatine and creatinine have been described in detail in a previous paper (9). All values given for creatine were corrected for GAA present in the samples and vice versa. To minimize spontaneous conversion of creatine to creatinine urine specimens were kept at 4° after collection and were analyzed with minimal delay. During the period in which 10 gm. of creatine were ingested daily, the urines were kept frozen until analyzed. Thymol was used as preservative, and analyses were done before bacterial growth was evident. All urines were extracted with chloroform after dilution to remove traces of protein before analysis for GAA. The GAA was synthesized by the method of Nencki and Sieber (10) and gave theoretical values for nitrogen on analysis.

#### CALCULATIONS

In previous studies on the same subject over a period of several months the fraction of the body creatine excreted as creatinine per day was found to be 0.0164 (6). Therefore, in estimating changes of body creatine, the average daily creatinine excretion, expressed as creatine, during the last 4 days of each period has been divided by

this factor. The average basal excretion of GAA for this subject was 100 mg. per day. In calculating the recovery of ingested GAA, this quantity has been subtracted from the daily excretion to give the excess GAA excreted. Creatine or GAA excreted as excess creatinine has been calculated by subtracting the average basal creatinine excretion of the subject, 1.96 gm. from the total daily excretion, all values for creatinine being expressed as the creatine equivalent. When heavy creatinuria was present, spontaneous conversion of creatine to creatinine during the interval between collection of urine and analysis may make this value somewhat higher than the actual value, but this increment is exactly balanced in the analytical procedure by the concomitant reduction in the values for creatine.

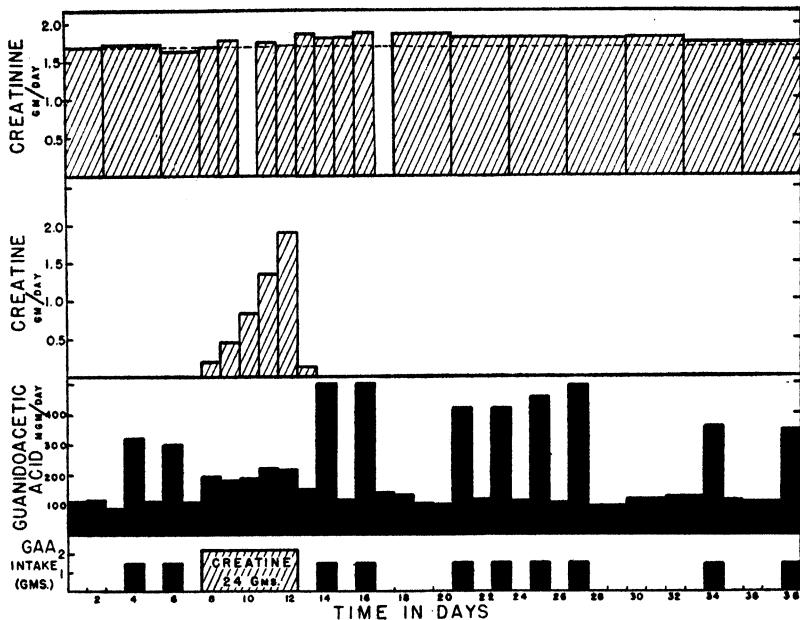


FIG. 1. TOLERANCE TO GUANIDOACETIC ACID before and after ingestion of creatine. The initial level of creatinine excretion is indicated by the dotted line.

## RESULTS

In figure 1 are shown the results of a series of GAA tolerance tests made before and after the ingestion of 24 gm. of creatine over a 5-day period. This experiment was designed to duplicate the previous study made in this laboratory in which a similar amount of creatine was given to the same subject after the tissue creatine had been labeled with N-15. A slightly smaller quantity, 19.4 as opposed to 22.6 gm., of creatine was retained than in the previous experiment. This may be explained by the fact that the subject had been on a diet free of creatine for a shorter period prior to the experiment. Since the previous studies had suggested a retardation of creatine synthesis during and following the ingestion of the non-isotopic creatine, it was ex-

pected that GAA given shortly after the creatine ingestion would appear in larger amount in the urine. Such was in fact the case; twice as much GAA in excess of the basal excretion appeared in the urine when tolerance tests were made after the creatine ingestion as before. But the results of subsequent tolerance tests were inconsistent in several respects with the concept that creatine synthesis was retarded. Successive tests made on the 9th to the 15th days following the creatine ingestion showed successively decreasing instead of increasing tolerance during the time when it would be expected from previous data that endogenous synthesis would have returned to initial values. In addition, the creatinine excretion remained elevated for as long as GAA continued to be ingested. From an initial value of 1.69 gm. the daily creatinine output was elevated to an average of 1.82 gm. during the period immediately following the creatine ingestion. From the 12th to the 20th day following the creatine ingestion this averaged 1.82 gm. per day. Previous data had shown that creatinine excretion and the tissue creatine from which it is derived would normally have returned almost to the initial value by the end of this latter period. Thus it is inferred that a large portion of the 9 gm. of GAA given in the tolerance tests subsequent to the creatine ingestion was methylated to form creatine.

Before testing this inference further, the subject's tissues were first saturated with creatine by the ingestion of 90 gm. over a period of 9 days. Of this quantity 33 gm. were retained. Creatine ingestion was then continued at the rate of 1 gm. per day. Even this small quantity taken in 60-mg. portions at hourly intervals was found sufficient to sustain a minimal but definite creatinuria. Serum creatine likewise was elevated to a value of 0.85 mg. per cent at the end of the 57th day, 8 hours after the last 60 mg. dose of creatine. Thus a portion of any creatine formed from exogenous GAA would be expected to appear in the urine. After the creatinuria had approached a basal quantity, 1.5 gm. of GAA were added to the 1.0 gm. of creatine on the 52nd, 54th and 58th days. As is shown in figure 2 on each occasion the excretion of creatine was significantly increased. On the 63rd day 2.5 gm. of GAA alone were substituted for the creatine ingested. Creatinuria persisted and gradually increased to over 0.5 gm. by the 8th day after starting the ingestion of GAA alone and averaged 0.62 gm. for the latter half of the period. The creatinine excretion did not fall during this time, which suggests that the creatine of the urine was not derived from the tissue depots. The average daily creatinine excretion from the 58th to the 62nd day, when creatine alone was ingested was 1.89 gm.; that during the last 13 days of GAA ingestion was 1.96 gm. Since the creatinuria was greater during this latter period, it is possible that a part of this apparent increase may represent spontaneous conversion of creatine to creatinine. In the presence of over 10 times as much urinary creatine on days 39 through 47, however, creatinine values did not exceed 1.97 gm. daily.

The constant ingestion of GAA with production of creatinuria withdraws methyl groups from the body. To determine whether this depletion of labile methyl was a factor limiting the quantity of creatine formed from the GAA, the dietary protein was restricted to 60 gm. per day during the first 11 days of GAA ingestion. Six gm. of DL-methionine were added to the diet during the last 2 days to provide a source of methyl groups. The creatinuria did not change during this latter period, the 72nd and 73rd days shown in figure 2.

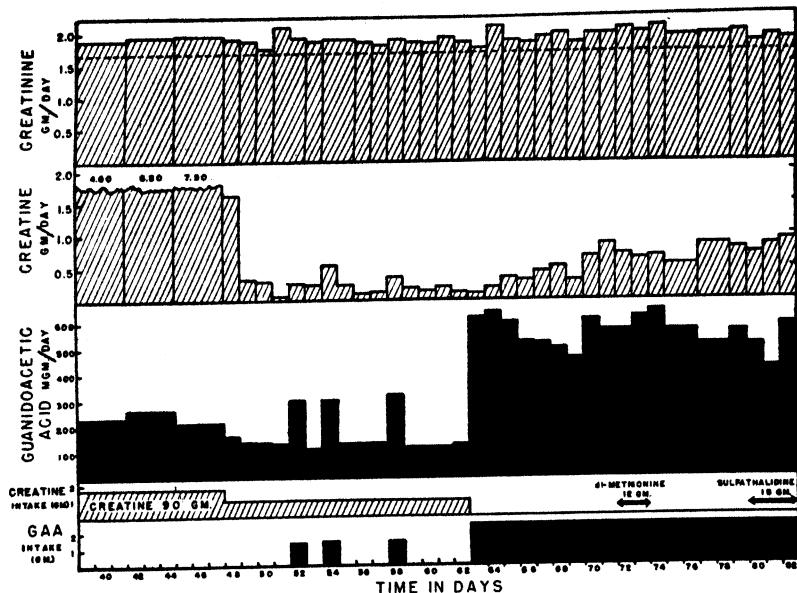


FIG. 2. PRODUCTION OF CREATINURIA from ingestion of guanidoacetic acid. The initial level of creatinine excretion is indicated by the dotted line. On the morning of the 58th day serum creatine was 0.85 mg.% and serum creatinine 1.4 mg.%.

TABLE I. RECOVERY OF INGESTED CREATINE AND GUANIDOACETIC ACID

	PERIOD			Total Days 1-82
	I Days 1-38	II Days 39-62	III Days 63-82	
Creatine ingested.....	24.0	104.0	0	128.9
Guanidoacetic acid ingested.....	16.8	4.9	50.0	77.7
Total intake.....	40.8	109.8	56.0	206.6
Creatine excreted.....	4.8	61.5	9.9	76.2
Guanidoacetic acid excreted in excess of basal excretion of 100 mg/day.....	4.4	2.4	10.2	17.0
Creatine excreted in excess of basal excretion of 1.69 gm/day.....	4.3	6.3	6.0	16.6
Estimated increase in body creatine based on creatinine excretion (see text).....	5.0	0.5	1.0	15.5
Total creatine and/or guanidoacetic acid accounted for:				
Total.....	18.5	79.7	27.1	125.3
Per cent.....	45	73	48	61
Grams per day of ingested creatine or guanidoacetic acid not accounted for.....	0.59	1.26	1.44	0.99

All values for creatinine and guanidoacetic acid are expressed as the creatine equivalent.

Since Bodansky (5) and Twort and Mellanby (11) had isolated from intestinal contents Gram-positive bacteria capable of rapidly destroying creatine, the subject took sulfathalidine for a 3-day period in an attempt, during GAA ingestion, to augment the creatinuria. This did not change, as can be seen during days 79 to 82 of the same figure.

In table 1 is given the over-all balance between ingested and excreted creatine and GAA, together with an estimation of changes in body creatine. The method of calculation is described above. Of the total intake 39 per cent cannot be accounted for or recovered. The study has been divided into three periods. In *Period I* (days 1-38, corresponding to fig. 1) during which 24 gm. of creatine were ingested and GAA tolerance tests were done, 45 per cent of the ingested compounds could not be accounted for. In *Period II* (days 39-62 of fig. 2) 105 gm. of creatine and 5 gm. of GAA were ingested. The greater part of the large quantity ingested during the first 9 days was excreted directly as creatine so that the percentage accounted for was high (73%). But of the 43.4 gm. of creatine actually retained only 31 per cent was accounted for. In *Period III* (days 63-82 of fig. 2), only GAA was ingested. Of the 45.8 gm. of GAA, expressed as creatine, which was not excreted directly as GAA, 9.9 gm. were excreted as creatine; there was an apparent increase in the tissue creatine of 1.0 gm., and 6 gm. of extra creatinine were excreted. Thirty-seven per cent of the retained GAA was accounted for. The fact that in the preceding period the recovery of creatine itself was incomplete suggests that the actual quantity of GAA converted to creatine may have been greater.

#### DISCUSSION

During the latter two periods of the above experiment the body creatine was at or close to the maximum value for the subject. An average of 1.26 gm. per day of creatine could not be accounted for during the second period, when creatine was ingested uninterruptedly. To account for this discrepancy on the basis of an inhibition of GAA methylation to creatine, there would have to be roughly a 65 per cent inhibition of the methylation of the 1.9 gm. of endogenous GAA synthesized per day. However, in the present study a creatinuria of from 0.5 to 0.9 gm. per day was produced by the ingestion of GAA under these conditions, and 37 per cent of the GAA not excreted as such could be accounted for as creatine or creatinine. When creatine itself was ingested in the preceding period, 31 per cent of the comparable amount which was retained could be accounted for. The calculations are certainly only approximations, but they suggest that there was no essential difference in the manner in which the two substances were metabolized. If GAA were diverted under these conditions to a metabolic pathway other than the formation of creatine, one would expect on comparing a period of creatine with one of GAA ingestion to recover much less of the GAA than of the creatine. The findings are consistent with a conversion of a major portion of the retained GAA to creatine.

The rise in GAA excretion which occurs when creatine is ingested can be explained on the basis of a competition by the two substances for renal tubular reabsorption (9). The creatinuria from GAA ingestion, however, cannot be explained on this

basis for two reasons. Administration of GAA during clearance studies did not reduce the reabsorption of creatine by the renal tubules (9). Moreover, under the conditions of the present experiment creatinine excretion increased or remained constant during the periods of GAA ingestion, suggesting that the body creatine was not decreased.

The metabolic fate of that portion of administered GAA or creatine which has not been accounted for by balance or isotope studies remains obscure. The answer may lie in the possibility emphasized by Bodansky (5) that a portion of the creatine transported to or formed in the liver is secreted with the bile and is thus subject to bacterial destruction in the intestines before reabsorption. In his experiments on rats he showed that after a single 100-mg. dose of GAA the creatine of the intestinal contents determined by the enzymatic method was increased five times above normal values at 12 hours and remained double the normal value at 24 hours. It seems possible that the small amounts of N-15 recovered in the urinary ammonia after feeding labeled creatine to rats (3) and to man (6) is derived from creatine destroyed in the gut. There is evidence suggesting that the liver serves as a primary reservoir for exogenous creatine before its more gradual transfer to muscle (6). With greater than physiological quantities, a larger proportion may thus be subject to excretion in the bile and subsequent bacterial destruction. That a larger quantity of N-15 is recovered in the urinary ammonia and urea on administering comparable quantities of GAA to man (6) might be explained by the fact that GAA must first be converted to creatine in the liver, whereas exogenous creatine may directly enter muscle. Since the organisms capable of destroying creatine which were described by Twort and Mel lanby (11) and by Bodansky (5) are anaerobes and possibly not inhibited by sulfonamides, the negative results with sulfathalidine in the present experiment is not conclusive.

During these experiments the maximum creatinine excretion averaged over any 5-day period following creatine ingestion was 2.00 gm. per day. Assuming that the subject excreted the same proportion of his body creatine as during the previous isotope experiments, or 1.64 per cent, this would correspond to a total body creatine of approximately 140 gm. This represents a 20 per cent expansion of the initial body creatine and appeared to be the maximum for the subject.

In 1921 Gibson and Martin (12) noted an increase in apparent creatinuria during one day in which GAA was fed to a child with muscular dystrophy. Similarly, in this laboratory we have produced creatinuria by feeding GAA to a patient with myotonia atrophica who had shown inability to retain administered creatine (13). In the present study creatinuria has been produced by administration of GAA to a normal subject. This is compatible with the concept that methyltestosterone produces its effect on creatine metabolism solely by accelerating the synthesis of guanidoacetic acid.

#### SUMMARY

In experiments during which creatine and guanidoacetic acid were ingested by a normal subject no evidence was obtained suggesting that methylation of guanidoacetic acid to form creatine is retarded when an excess of creatine is present. It was

possible to produce a substantial creatinuria by the administration of guanidoacetic acid alone, thus simulating the effect of methyltestosterone on creatine metabolism. This suggests that the sole action of methyltestosterone on creatine metabolism is in acceleration of guanidoacetic acid synthesis.

The author is indebted to Dr. J. P. Peters and to Dr. H. D. Hoberman for valuable advice and criticism.

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# EFFECT OF MAMMALIAN (POSTERIOR LOBE) PITUITARY EXTRACT ON WATER BALANCE OF FROGS WHEN PLACED IN DIFFERENT OSMOTIC ENVIRONMENTS<sup>1</sup>

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THE frog lives in a hypotonic environment, while maintaining body fluids of considerable osmotic activity. There is an osmotic gradient between the external water and the internal body fluids, which tends to force water into the tissues. At the same time, the frog skin is an important respiratory membrane which must be permeable to gases. This also makes impermeability to water improbable. The frog skin is in fact somewhat permeable to water, which continually flows into the frog's tissues (1).

The continual dilution of the internal environment is amended by excretion of a large volume of very dilute urine (2). Osmoregulation is attained by a balance between these two processes. Under normal conditions the skin offers a resistance to the inward passage of water (3), which spares the necessary osmotic work otherwise imposed on the kidney.

Several factors are known to disturb the water balance of frogs living in water. Low temperature (4), anaesthesia (5, 6), destruction of part or all of the central nervous system (7), and flaying (5) all cause an increase in body water.

Large doses of mammalian posterior lobe pituitary extract cause summer frogs to gain about 20 per cent in weight in 3 hours. The weight then declines to normal or lower in about 12 hours (8). The hormone responsible is probably different from either the vasopressor or oxytocic principles, although associated with the latter. The hormone is more abundant in the lower vertebrates than in mammals, and has been described in extracts of crustacean eye-stalks (9, 10).

Pituitary extract increases the rate of weight gain of frogs in which the escape of urine is prevented by anal ligatures (11). Therefore the effect is due (at least partially) to an increased rate of influx of water compared with normal. The extract seems to lower the resistance of the skin to the inward diffusion of water. Reports also exist which indicate that pituitary extract inhibits water loss in the frog (12).

This investigation concerns the effect of posterior lobe pituitary extract on the water balance of frogs when placed in different osmotic environments.

## METHODS

Frogs (*R. pipiens*) were taken at random from storage tanks. They were weighed roughly to the nearest 0.1 gm. and arranged in order of increasing weight. Alternate frogs were then assigned to experimental and control groups. This bal-

Received for publication February 23, 1949.

<sup>1</sup> This work was supported by a grant from the National Research Council of Canada.

anced the body weights between the groups, and eliminated a source of variation. There were usually 10 frogs in each group.

In some experiments purse string sutures were tied through the perianal skin. This usually prevented the escape of urine. Occasionally a leak developed, and the frog was discarded from the experiment. By this procedure the urine was weighed with the frog, and the results obtained then referred only to the rate of exchange of water through the skin.

A commercial mammalian posterior lobe extract was used throughout (Pituitrin S., Parke, Davis). This extract contains both vasopressor and oxytocic principles. The dosage was chosen to give a maximum increase in body weight, according to the dosage-response curve of Boyd and Young (13). This was 1.0 I.U. per 10 gm. of body weight, by injection in the dorsal lymph sac.

The osmotic environment was varied by placing the frogs in sucrose solutions of three different concentrations. Sucrose was used for two reasons: 1) since frog skin actively transports a number of ionized substances, a non-electrolyte was desirable; 2) the frog skin is probably relatively impermeable to sucrose.

Three levels of osmotic activity were used: hypotonic, isotonic and hypertonic. The hypotonic solution used was simply distilled water. In this case the osmotic gradient forces water inward, and is equal to the osmotic activity of the frog tissues. The osmotic activity of the internal environment of the frog is about 0.43°C. (14) corresponding to a pressure gradient of 5.2 atmospheres.

Isotonic sucrose was taken to be 0.23 molar. In this solution, the osmotic gradient across the frog's surface is approximately zero.

The hypertonic solution of sucrose was made up to 0.46 molar, just twice the isotonic concentration. This approximately reverses the normal osmotic gradient, and forces water outward from the tissues into the solution. The frogs were kept in 100 cc. of the appropriate solution, in individual jars with perforated tops. The size of the jars was such that a frog in a sitting position could keep the external nares at the surface of the solution.

Weighings were performed at suitable intervals after the beginning of the experiment. For weighing, a frog was removed from the solution, and dried lightly on a towel. The urine was expressed in the case of a normal frog. If an anal ligature had been placed, it was inspected for leaks. The frog was then weighed to the nearest 0.1 gm. on a beam balance, and replaced in the solution.

#### RESULTS

The weight changes observed were expressed as percentages of the original weight of the frog at the beginning of the experiment. The mean percentage weight increases are plotted against time in figures 1 to 6.

Figure 1 shows the weight-time curves for frogs in distilled water, with and without the injection of pituitary extract. The extract caused a typical transient weight gain, followed by a return toward normal. The controls lost weight slightly, probably related to the handling of the frogs.

Figure 2 shows the result of the same experiment, with anus-ligated frogs. The controls (ligature only) gained weight at the rate of 1.8 per cent of body weight per

hour. This represents the normal rate of influx of water into the frogs and the normally equal rate of excretion of urine. Pituitary extract increased this normal rate of

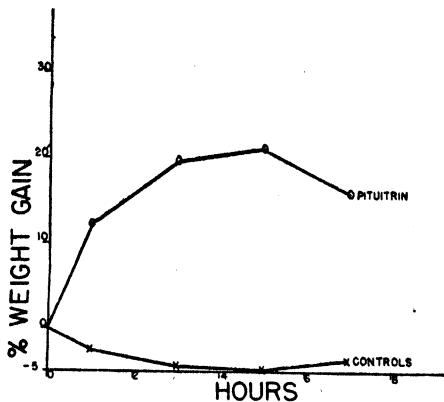


Fig. 1. MEAN WEIGHT CURVES FOR NORMAL FROGS in water, with and without the injection of 1.0 I.U. of mammalian pituitary (posterior lobe) extract per 10-gm. body weight.

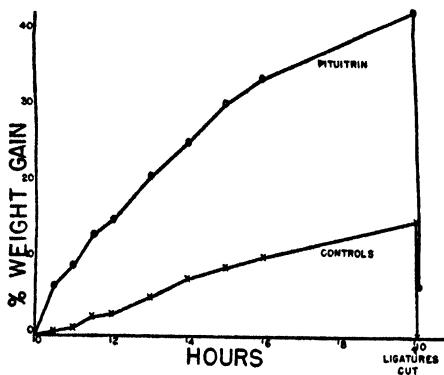


Fig. 2. MEAN WEIGHT CURVES FOR FROGS WITH ANAL LIGATURES, in water, with and without the injection of 1.0 I.U. of mammalian pituitary (posterior lobe) extract per 10-gm body weight.

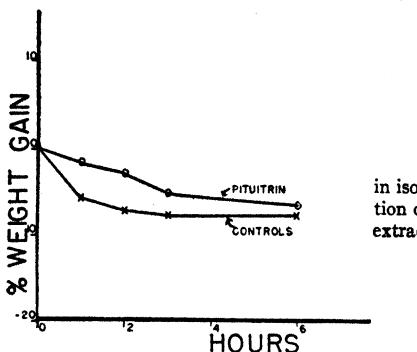


Fig. 3. MEAN WEIGHT CURVES FOR NORMAL FROGS in isotonic sucrose solution, with and without the injection of 1.0 I.U. of mammalian pituitary (posterior lobe) extract per 10-gm. body weight.

influx to about 7.5 per cent of body weight per hour. When the ligatures were cut, there was a prompt and marked loss of urine.

Figure 3 shows the weight-time curve for frogs in 0.23 molar sucrose solution

(isotonic), with and without pituitary extract. In the controls there was a rapid loss of weight in the first 2 hours, after which the weight became constant. The pituitary

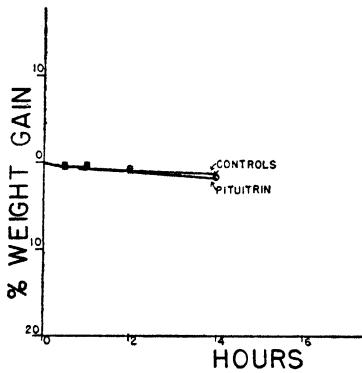


Fig. 4. MEAN WEIGHT CURVES FOR FROGS WITH ANAL LIGATURES, in isotonic sucrose solution, with and without the injection of 1.0 I.U. of mammalian pituitary (posterior lobe) extract per 10-gm. body weight.

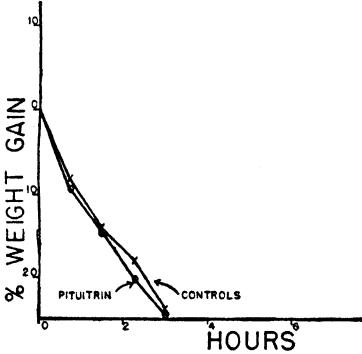


Fig. 5. MEAN WEIGHT CURVES FOR NORMAL FROGS in hypertonic sucrose solution, with and without the injection of 1.0 I.U. of mammalian pituitary (posterior lobe) extract per 10-gm. body weight.

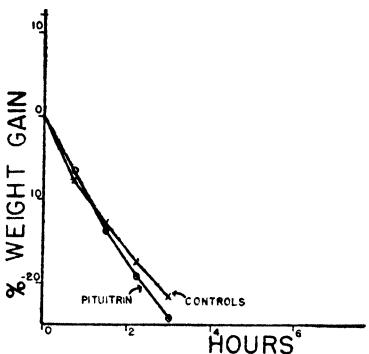


Fig. 6. MEAN WEIGHT CURVES FOR FROGS WITH ANAL LIGATURES, in hypertonic sucrose solution, with and without the injection of 1.0 I.U. of mammalian pituitary (posterior lobe) extract per 10-gm. body weight.

extract retarded this weight loss, although the final result was the same within experimental error. Figure 4 shows the result of the same experiment with anus-ligated frogs. In this case there was no appreciable weight loss, and no change due to pituitary extract.

Figure 5 shows the result when the normal osmotic gradient was reversed by placing the frogs in 0.46 molar sucrose solution. The weight loss was rapid, about 8.5 per cent of body weight per hour. Injection of pituitary extract caused no significant difference in the rate of weight loss. Figure 6 shows the result of the same experiment in anus-ligated frogs. The curves are practically identical with those of figure 5, i.e. neither anal ligature nor pituitary extract changed the rate of weight loss of frogs in 0.46 molar sucrose solution.

#### DISCUSSION

Consider first the water exchanges of frogs when subjected to the three levels of ambient osmotic activity: *a*) hypotonic, *b*) isotonic and *c*) hypertonic.

*Hypotonic.* For a normal frog in water, a steady state is attained when the influx of water through the skin is balanced by the excretion of urine. If the anus is blocked by a ligature, the weight increase over the next few hours is a measure of the normal influx and loss of water through the frog. From figure 2, this is found to be approximately 1.8 per cent of body weight per hour. This rate depends on 2 factors: *1*) on the magnitude and direction of the osmotic gradient between the external solution and the tissues, and *2*) on the resistance of the intervening tissues to the flow of water.

*Isotonic.* When frogs are transferred from water to isotonic sucrose solution, they lose weight for about 2 hours and then establish a new steady state (fig. 3). If the escape of urine is prevented, this adjustment does not take place (3). In the absence of an osmotic gradient the influx of water ceases, as is obvious from figure 4, but the excretion of urine continues for a short time, causing some weight loss as shown in figure 3.

*Hypertonic.* When frogs are transferred from water to 0.46 molar sucrose solution, the normal osmotic gradient is approximately reversed. There results a rapid weight loss of about 8.5 per cent of body weight per hour, which is not modified by anal ligature. This loss must therefore take place through the skin, assuming no loss through gastro-intestinal tract. The rate of outflow of water under these conditions is about 4.7 times the inflow when the gradient is in the normal direction, as shown by comparing figures 2 and 6. Since the osmotic gradients are approximately equal and opposite, the difference must lie in the resistance of the tissues to the flow of water in opposite directions. The greater resistance to inflow is of obvious advantage to the frog since it reduces the amount of urine which would otherwise have to be excreted. The exact nature of the resistance is unknown, although speculative hypotheses have been proposed (1).

Consider secondly the effect of injection of pituitary extract on the water exchanges, under the 3 sets of conditions: *a*) hypotonic, *b*) isotonic and *c*) hypertonic.

*Hypotonic.* With frogs in water, pituitary extract causes a rapid increase in body weight, up to 20 per cent in 5 hours. The initial rate of weight gain is about 10 per cent per hour, approximately five times the normal rate of flow of water through the frog. The magnitude of the increase in flow due to pituitary extract could not be due to oliguria alone, since it greatly exceeds normal urine output. The extract therefore decreases the resistance of the surface of the frog to the inflow of water.

A comparison of figures 1 and 2 showing the effect of pituitary extract demonstrates

two phenomena. First, as noted above, it causes a greatly increased uptake of water, which exceeds normal urinary output by some five times. Secondly, since in the first 3 hours the effect of pituitrin was approximately the same in normal frogs and those with anal ligatures, it appears that this drug completely inhibits urinary water loss in the doses used. Pituitrin in effect prevents water loss through urine. This oliguric effect is probably renal in origin, because pituitary extract causes a marked reduction in the number of active glomeruli in the frog kidney, and consequent anuria (15). It seems that the uptake of water after pituitary extract is due to a combination of decreased resistance to the influx of water, and decreased excretion of urine.

*Isotonic.* In isotonic solution, pituitary extract delays the excretion of urine (fig. 3) but has no effect on the body weight when the escape of urine is prevented (fig. 4). An osmotic gradient favoring influx of water is necessary for the effect of pituitary extract to become apparent.

*Hypertonic.* When the osmotic gradient is reversed, pituitary extract has no effect on the already high rate of outflow of water through the skin.

#### SUMMARY

The frog offers a resistance to the influx of water from hypotonic solution, as compared to the outflow of water when the osmotic gradient is reversed. This resistance is greatly diminished by pituitary extract (posterior lobe). An oliguria is also produced by mammalian posterior pituitary extract, which contributes to the net gain in weight by water uptake from hypotonic solution.

The author is indebted to Mr. W. Sped of the Parke, Davis Company, for generous supplies of Pituitrin S.

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# EFFECT OF THE BLOOD GLUCOSE LEVEL ON THE SECRETION OF THE ADRENAL CORTEX

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**I**NVESTIGATIONS in recent years have shown that the adrenal cortex plays an important rôle in maintaining the resistance of the animal organism to stress (1, 2). In view of this, any study which contributes to an understanding of the mechanism which regulates the secretion of this endocrine organ assumes significance. It is now generally accepted that the activity of the adrenal cortex is controlled by the adrenocorticotropic principle, which is secreted by the anterior pituitary.<sup>1</sup> The mechanism of the secretion of the adrenal cortex is, therefore, mainly associated with the factors influencing the release of the adrenocorticotropic hormone from the anterior pituitary. Recently, it has been found that epinephrine plays an important rôle in the control of adrenal cortical activity and that this control is mediated through the anterior pituitary by bringing about a release of the adrenocorticotropic hormone (1, 2). The manner by which epinephrine produces this effect is not known.

Since epinephrine and the blood glucose level are so closely interrelated, experiments have been performed to determine whether the blood glucose level *per se* would influence the activity of the adrenal cortex. Alterations in the cholesterol content of the adrenals have been used as a measure of elaboration of cortical hormones by the adrenal cortex. Evidence has recently been obtained that a relationship exists between the amount of cholesterol, present in the adrenal, with the gland's secretory activity (1, 2). The nature and character of this relationship are not well understood. The changes in the cholesterol content of the adrenal induced by hyperglycemia or hypoglycemia are presented.

## PROCEDURE

White adult male rats of the Wistar and Sprague-Dawley strains, weighing between 225 and 280 grams were employed. The animals were starved overnight but allowed water.

*Oral Administration of Glucose.* The rats were intubated with a catheter and 2 ml. of a 50 per cent glucose solution were introduced orally by means of a hypodermic syringe into the experimental and 2 ml. of distilled water into the control animals. After certain time intervals, the animals were killed by the guillotine method. The adrenal glands were immediately dissected, weighed and analyzed for cholesterol according to the method of Schoenheimer and Sperry (4). The total cholesterol was

Received for publication March 3, 1949.

<sup>1</sup> Deane, Shaw and Greep have presented evidence that at least in the rat the secretion of the electrolyte-regulating hormones of the adrenal gland is not under the control of the anterior pituitary (3).

determined in each case and the amount of cholesterol is reported as mg./100 mg. of fresh wet adrenal tissue. Blood sugar determinations were carried out according to the procedure of Somogyi (5).

It was found that handling and intubation of the animals led to an 'alarm' reaction, as indicated by a decrease in adrenal cholesterol. For this reason, it was essential to accustom the animals to the experimental conditions before being used. This was done by passing a fine rubber catheter into the stomach twice daily for at least eight days. Only healthy, growing rats are suitable for studies of adrenal cholesterol changes inasmuch as inanition or infection will markedly influence the adrenal cholesterol levels. The animals were kept as much as possible in an environment of 24° to 26° C.

*Administration of Insulin.* The experimental animals were injected subcutaneously with 0.5 I.U. of insulin while the control animals were injected subcutaneously

TABLE I. CHANGES IN THE CHOLESTEROL CONTENT OF THE ADRENALS AND IN THE BLOOD SUGAR LEVELS IN NORMAL RATS AFTER ORAL ADMINISTRATION OF 2 ML. OF 50% GLUCOSE SOLUTION

NO. OF ANIMALS	TYPE OF EXPERIMENT	TIME OF DEATH AFTER INJECTION	WT. OF FRESH ADRENAL GLANDS	CHOLESTEROL CONTENT OF 100 MG. OF FRESH ADRENAL TISSUE		BLOOD SUGAR LEVEL IN MG. % AT TERMINATION
				min.	mg.	
18	Control 2 ml. H <sub>2</sub> O	1	35	3.44 ± 0.10		72
12	Glucose	30	33	4.91 ± 0.16		81
12	Glucose	60	36	3.38 ± 0.18		99
12	Glucose	120	36	3.83 ± 0.11		90
7	Glucose	240	34	3.57 ± 0.09		
8	Glucose	300	36	3.21 ± 0.11		70
4	Glucose	360	34	3.70 ± 0.07		

<sup>1</sup> The animals were killed at the same time intervals as the glucose injected rats.

with 0.25 ml. of saline solution. The adrenal cholesterol and blood glucose levels were determined ninety minutes after administration.

#### RESULTS AND DISCUSSION

Table I illustrates the mean cholesterol changes in the adrenals as well as the mean blood sugar changes determined simultaneously in rats after oral administration of 2 ml. of a 50 per cent glucose solution in water. Oral administration of glucose produced a distinct increase in the adrenal cholesterol and an elevation of the blood sugar level half an hour after administration. Between 30 to 60 minutes, there is a sharp decline in the adrenal cholesterol content to normal levels. The increase in the adrenal cholesterol level, observed at thirty minutes, was significant inasmuch as the lowest values for cholesterol found at that time were definitely greater than the highest values obtained in the control animals. The rapidity of the rise and decline of the adrenal cholesterol after the administration of glucose is rather surprising.

Elmadjian, Freeman and Pincus (6) found that oral administration of glucose (1 ml. of a 50% solution) to rats produced a lymphocytopenia within one to two hours

after administration. We also observed a decrease in lymphocytes after oral glucose administration at a time at which the cholesterol levels of the adrenals were normal. The fall in lymphocytes, which is supposed to indicate a stimulation of the adrenal cortex, may possibly be due to the actual outpouring of adrenocortical hormones occurring within 30 to 60 minutes after glucose administration. The decline in adrenal cholesterol and the release of adrenocortical hormones at a time during which there still exists a pronounced hyperglycemia may be explained as follows.

a) The hyperglycemia causes a stimulation of the secretion of insulin, which according to Vogt (7), may directly stimulate the release of the adrenocorticotropic hormone from the anterior pituitary.

b) The inhibition of the adrenal cortex, as indicated by the increased adrenal cholesterol content, leads to a lowered concentration of adrenocortical hormones in the blood which, according to Sayers and Sayers (8), causes a stimulation of the liberation of adrenocorticotropic hormone from the anterior pituitary.

TABLE 2. MEAN CHANGES IN THE CHOLESTEROL CONTENT OF THE ADRENALS AND IN BLOOD SUGAR LEVELS IN NORMAL RATS AFTER SUBCUTANEOUS INJECTION OF 0.5 I.U. INSULIN

NO. OF ANIMALS	TYPE OF EXPERIMENT	TIME OF DEATH AFTER INJECTION	WT. OF FRESH ADRENAL GLANDS	CHOLESTEROL CONTENT OF 100 MG.M. OF FRESH ADRENAL TISSUE	BLOOD SUGAR LEVEL IN MG. % AT TERMINATION
				mg.	
5	Control	90	35	$3.82 \pm 0.03$	72
	Uninjected				
12	Control	90	32	$3.59 \pm 0.19$	66
	0.25 ml. Normal saline				
18	Insulin	90	32	$2.04 \pm 0.11$	20

Abelin (9) observed that, 7 to 8 hours after oral glucose administration (1 gm./100 gm. of body weight) to rats, a reduction of about 25 per cent in the adrenal cholesterol occurred. In repeating Abelin's experiments, similar results were obtained. The adrenal cholesterol content apparently increased within a short period after oral administration of glucose, then rapidly fell to a normal level while hyperglycemia still persisted and stayed at a normal level for several hours, during which time the blood sugar level gradually became normal. After 8 hours, a lowering in the adrenal cholesterol content was observed.

Since the initial hyperglycemia led to an increase of the adrenal cholesterol content, investigation was directed toward the determination whether hypoglycemia would effect a lowering of the adrenal cholesterol.

As can be seen from table 2, insulin-induced hypoglycemia effected a stimulation of the adrenal cortex as indicated by the lowered adrenal cholesterol level. This finding is in agreement with that of Gershberg and Long (10) who observed a fall in the adrenal ascorbic acid and with that of Vogt (7) who found a depletion in the adrenal lipids of rats following insulin injection.

The findings reported in this paper show that hyperglycemia produced by oral glucose administration leads to an inhibition of the release of the hormones from the

adrenal cortex, as indicated by an increase in the adrenal cholesterol content. The data given support the assumption that the initial hyperglycemia leads to a diminished release of epinephrine by the adrenal medulla with a correspondingly lessened release of the adrenocorticotropic principle from the anterior pituitary. In hypoglycemia, the reverse takes place. Whether or not this effect of the blood glucose level on the secretion of the adrenal cortex is directly mediated through the anterior pituitary or indirectly through the adrenal medulla is not as yet established.

#### SUMMARY

Hyperglycemia induced by oral administration of glucose in rats causes, within 30 minutes, an elevation of the adrenal cholesterol level indicating an inhibition of the adrenal cortex. Hypoglycemia induced by insulin injection in rats produces a decrease of the adrenal cholesterol level 90 minutes after administration indicating a stimulation of the adrenal cortex.

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# ACTION OF VITAMIN P ON THE STABILITY OF CONNECTIVE TISSUE GROUND SUBSTANCE<sup>1</sup>

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**W**HEN vitamin P was discovered, its function was said to be the regulation of the permeability and strength of capillaries (1). Since then, all the work which has been done in this field has been focused on capillaries, and the possibility that this vitamin might be concerned with the control of the permeability of other tissues has received scant attention.

In a previous communication (2), the author showed that rutin, a flavonol type of vitamin P, inhibited the diffusion of testicular hyaluronidase and control saline solutions injected intradermally in rats. Since the spread of the control solution could conceivably be associated with the activation of endogenous hyaluronidase, no conclusions could then be made as to whether rutin acted directly by inhibiting hyaluronidase, or indirectly by some action on the substrate. The probability that the latter was the case was suggested to the author by the findings of Griffith and Lindauer (3), that rutin prevented cerebral hemorrhage in hypertensive patients with increased capillary fragility. Although their study was stimulated by Paterson's (4) suggestion that cerebral hemorrhage developed on the basis of rupture of intimal capillaries, such a mechanism has never been clearly demonstrated in this type of vascular accident. It is more likely that cerebral hemorrhage is due to direct rupture of the whole thickness of the artery wall as a consequence of arteriosclerotic weakening combined with increased arterial blood pressure. This suggests that rutin acts on the entire vascular apparatus rather than solely on the capillaries. Gorev and Smirnova-Zamkova (5) have shown that the pericapillary sheath, the media and externa of arteries, and the connective tissue in general, all have in common, argyrophilic ground substance. It therefore appeared that the strengthening effect of rutin on the capillaries and arteries might be referable to a stabilizing effect on the ground substance which they contain. If such were the case, the stabilization of the generalized system of ground substance by rutin should be non-specific and demonstrable against spreading agents other than hyaluronidase. To check this hypothesis, rutin was tested against a potent, long-acting, synthetic spreading factor, azoserum. The studies were then extended to other types of vitamin P.

## PROCEDURE

A 6.5 per cent solution of rutin<sup>3</sup> was prepared in 0.2N sodium hydroxide. The pH, as measured in the Beckman meter, was 9.4. On the addition of 1.25 cc. N

Received for publication November 15, 1948.

<sup>1</sup> This study was aided by a grant from the Cooper Fund, Faculty of Medicine, McGill University.

<sup>2</sup> Fellow in Medical Sciences, American National Research Council.

<sup>3</sup> Furnished by the F. W. Horner Co., courtesy Dr. L. Mitchell and Mr. G. Ling.

hydrochloric acid to 20 cc. rutin solution, a creamy emulsion formed whose *pH* was 8.4. A control sodium hydroxide solution of similar *pH* was also prepared. The other types of vitamin P, being water soluble, were injected as aqueous solutions. All injections of vitamin P were intraperitoneal, and preceded the spreading factors by 40 minutes to 4 hours.

The spreading solutions consisted of azoserum (6) as prepared, and testicular hyaluronidase<sup>4</sup> (2 mg. per injection), and physiological saline. They were colored by adding one third of their volume of doubly filtered Higgins india ink. The injection mass in each case was 0.2 cc. The active spreaders were injected into the right flank, the control saline into the left. An attempt was made to place all injections at the same relative point in the flanks, midway between the insertion of the upper and lower extremities, and 4 cm. from the mid-dorsal line. If an appreciable amount of the injection leaked out superficially or went too deeply, the animal was immediately killed and discarded. Twenty hours after the intradermal injections, the animals were killed and skinned. The skins were mounted with the inner side up. The inked areas, which were clearly delineated, were outlined and transposed onto cellophane paper and the enclosed areas measured in square inches by a planimeter. The results were analyzed by comparing the difference between means with the standard error of the difference, and by the method of Analysis of Variance. Reduction of spreading by less than 15 per cent was arbitrarily considered to be insignificant.

#### OBSERVATIONS

*Effect of Rutin on Diffusion of Azoserum.* Rutin administered to rats<sup>5</sup> in single doses of 65 mg. (1.0 cc.) 4 hours before the spreading factor, resulted in inhibition of the diffusion of both the azoserum and the control saline to a degree which was highly significant statistically (table 1). Inspection of the means reveals that the control *pH* solution failed to exert any significant effect.

*Effects of Hesperedin Methyl Chalcone<sup>6</sup> and Epicatechin<sup>6,7</sup> on Diffusion of Hyaluronidase.* One hour before the spreading factor was injected into the rats<sup>8</sup> the above types of vitamin P were given in doses of 100 mg. (1.0 cc.) and 10 mg. (0.25 cc.) respectively. Diminution in the areas of spread of both hyaluronidase and control saline solutions failed to occur. Negative results were also observed in guinea pigs when the spreading factor was injected 40 minutes after the second of two 5 mg. doses of epicatechin given 6 hours apart. The guinea pigs were used in addition to rats since the vitamin P activity of catechins was first described in this species. Epicatechin was used in a small dosage because it was claimed to be the most potent type of vitamin P (7). Possibly larger doses might have been effective.

*Effects of Multiple Injections of Esculetin on Diffusion of Azoserum and Hyaluronidase.* The results in experiment 2 suggested that some types of vitamin P might require extended administration before their effects became apparent. Single

<sup>4</sup> Purchased from the Treemond Co., N. Y. The potency was listed as 100 T.R.U./mg.

<sup>5</sup> Male albinos, 275-325 gm.

<sup>6</sup> Furnished by the Hoffman-Laroche Co., courtesy Mr. P. Blanc.

<sup>7</sup> The catechin was a mixture of epimers containing 35 per cent epicatechin.

<sup>8</sup> Female albinos, 225-300 gm.

TABLE I. EFFECTS OF RUTIN ON THE AREAS OF SPREAD<sup>1</sup> OF INKED AZOPROTEIN AND OF CONTROL SALINE SOLUTIONS

STRAIGHT CONTROL		NaOH CONTROL		RUTIN IN NaOH	
Azoprot.	Saline	Azoprot.	Saline	Azoprot.	Saline
3.86	0.60	3.40	0.80	1.90	0.30
2.60	0.80	3.40	0.70	1.80	0.40
4.00	0.53	3.20	0.64	4.00	0.50
3.50	0.70	3.85	0.55	1.76	0.40
3.50	0.60	3.00	0.65	2.63	0.45
3.05	0.60	2.35	0.40	1.93	0.30
3.83	0.75	4.00	0.60	2.57	0.43
2.72	0.60	2.96	0.70	1.65	0.44
2.94	0.70	3.45	0.65	1.84	0.45
4.20	0.68	4.30	0.70	2.34	0.43
3.02	0.69	4.20	0.50	2.07	0.30
2.91	0.58	3.77	0.64	1.50	0.40
3.35	0.83	3.41	0.60	1.92	0.30
3.20	0.70			1.62	0.23
4.93	0.70			1.60	0.33
				2.72	0.43
				1.82	0.44
$\bar{X}$	3.44	0.67	3.48	0.62	2.10
					0.38
				AZOPROT.	SALINE
Mean difference				$1.34 \pm 0.22^2$	$0.29 \pm 0.026^2$
Variance ratio	Obs.			$30.0^2$	$104.0^2$
		0.01 sig.		7.56	7.56

<sup>1</sup> In square inches.<sup>2</sup> Highly significant.TABLE 2. EFFECTS OF ESCULIN ON THE DIFFUSION OF HYALURONIDASE, AZOPROTEIN, AND SALINE<sup>1</sup>

	CONTROLS			ESCU LIN		
	H'ase	Azoprot.	Sal.	H'ase	Azoprot.	Sal.
Range.....	1.2-3.0	2.17-3.72	0.4-0.8	1.23-3.5	1.84-3.1	0.20-0.63
N.....	18	15	33	18	15	33
$\bar{X}$ .....	2.23	2.96	0.548	1.69	2.38	0.465
				H'ase	Azoprot.	Saline
Mean difference				$0.54 \pm 0.19^2$	$0.58 \pm 0.17^2$	$0.083 \pm 0.025^2$
Variance ratio	Obs.			$10.2^2$	$12.32^2$	$11.1^2$
		0.01 sig.		7.44	7.68	7.04

5 mg. doses of esculin<sup>9</sup> (1.0 cc.) were given to rats<sup>10</sup> daily for 6 days, prior to the spreading factors. The subsequent diffusions of azoserum, hyaluronidase and control saline solution were all inhibited to a highly significant degree (table 2).

*Effects of Vitamin P Deficiency on Permeability of Ground Substance.* In the experiments listed so far, the various types of vitamin P were administered to animals on normal diets<sup>11</sup> and the results could be interpreted as pharmacological rather than vitaminic. It was therefore decided to determine if the state of tissue permeability was altered in vitamin P-deficient rats. Unfortunately, gray oats, one of the major constituents of a truly vitamin P-free diet (8), was not available locally so that this diet could not be used. As an alternative procedure, advantage was taken of Parrot's finding (9) that turnips contained a substance with the properties of antivitamin P. Two hundred gamma of an alcoholic extract of turnip were said to produce a drop in capillary resistance which could be prevented by 20 gamma of catechin. It was claimed that the turnip factor accelerated the *in vitro* oxidation of ascorbic acid, whereas vitamin P substances inhibited this reaction. Since the action of the turnip factor was opposed to that of vitamin P both *in vivo* and *in vitro*, it was called antivitamin P.

Forty-five of a group of 60 male white albino rats weighing from 180 to 210 gm. were put on an antivitamin P regime by maintaining them on a diet of turnips and water *ad lib.*, for 14 days. The remaining 15 rats served as normal diet controls. By the 12th day, 8 of the turnip group and 1 of the control group 'ad died. At this time, the turnip group was divided into 3 sub-groups. One sub-group was given 50 mg. ascorbic acid intraperitoneally on the 12th and 13th days. Another sub-group was given 5.0 mg. esculin intraperitoneally on the same days, and the third sub-group was not treated. On the 14th day intradermal injections of 0.2 cc. inked azoprotein and saline were made in all animals and they were then treated as previously.

#### RESULTS

The most striking finding (table 3) was the marked increase in diffusion of both azoprotein and saline in the rats on the turnip diet. With the amount of esculin used, the increased spread of the azoprotein was moderately but significantly reduced. Esculin had no significant effect on the enhanced diffusion of saline. Ascorbic acid failed to inhibit the increased spread of both azoserum and saline.

#### DISCUSSION

The results indicate that at least 2 types of vitamin P, rutin and esculin, are capable of hindering the diffusion through connective tissue of enzymatic and non-enzymatic spreading factors as well as inert saline-ink mixtures. Since the retardation of diffusion appears to be non-specific, it is probable that vitamin P does not act directly on the substances injected into the skin but rather, it decreases the permeability of the connective tissue in a non-specific way. When vitamin P

<sup>9</sup> Purchased from the Mercantile Import and Export Co., N.Y.

<sup>10</sup> Hooded males, 150-200 gm.

<sup>11</sup> Purina Fox chow for rats. Various greens for guinea pigs.

deficiency was induced, the permeability of the connective tissue was markedly increased and this was partially reversed by esculin. It has been shown that hyaluronidase spreads through isolated dead skin (10). Its spread must therefore be largely independent of the circulation and must occur directly through the connective tissue ground substance. Thus vitamin P appears to be one of the factors which governs the strength and permeability of ground substance. As was mentioned previously, Gorev and Smirnova-Zamkova (5) have shown that the ground substance present in connective tissue is only part of a diffuse system of this material and that it is also present in the media and adventitia of arteries. Bensley (11) has also identified it in the intima of arteries. Thus by acting on the ground sub-

TABLE 3. EFFECTS OF ESCULIN AND OF ASCORBIC ACID ON THE INCREASED DIFFUSION OF AZOPROTEIN AND SALINE IN RATS FED TURNIPS (ANTIVITAMIN P)

	REGULAR DIET		TURNIP DIET		TURNIP DIET + ESCULIN		TURNIP DIET + ASCORBIC ACID	
	Azop.	Sal.	Azop.	Sal.	Azop.	Sal.	Azop.	Sal.
Range.....	2.25-4.30	0.37-0.85	3.92-6.50	0.80-1.67	3.16-5.24	0.75-1.55	4.04-5.23	0.90-1.95
N.....	II	II	9	9	II	II	9	9
$\bar{X}$ .....	3.32	0.54	5.07	1.24	4.23	1.00	4.45	1.18
Mean difference			TURNIP DIET VS. REGULAR DIET		TURNIP DIET + ESCU LIN VS. TURNIP DIET		TURNIP DIET + ASCORBIC ACID VS. TURNIP DIET	
			Azop.	Sal.	Azop.	Sal.	Azop.	Sal.
			1.75 $\pm$ 0.35 <sup>3</sup>	0.70 $\pm$ 0.11 <sup>3</sup>	0.85 $\pm$ 0.35 <sup>3</sup>	0.24 $\pm$ 0.125	0.62 $\pm$ 0.32	0.06 $\pm$ 0.14
Variance ratio		Obs.	25.6 <sup>3</sup>	47.3 <sup>3</sup>	5.8 <sup>3</sup>	3.82	3.67	—
		o.o1 sig.	8.28	8.28	4.4	4.4	4.49	—

<sup>1</sup> Figures in sq. inches.    <sup>2</sup> Significant.    <sup>3</sup> Highly significant.    <sup>4</sup> Below arbitrary level of significance.

stance of vascular walls, vitamin P appears to be a factor which determines the strength of vessel walls. Such an action on the 3 layers of the artery, rather than a capillary mechanism may account for the decreased incidence of cerebral hemorrhage which Griffith and Lindauer (3) observed following administration of rutin to patients with hypertension and increased capillary fragility.

The ability of vitamin P to increase capillary resistance has never been correlated with an action on any particular layer of the capillary wall. Several investigators (12-18) have shown that in pure scurvy uncomplicated by vitamin P deficiency, the capillary resistance is normal. Vitamin P is therefore able to act on a capillary layer which is not damaged in scurvy. This precludes the interendothelial cement whose production is said to be arrested in scurvy (19). Since the pericapillary sheath is believed to be a condensation of ordinary connective

tissue (19) the finding that vitamin P increases the resistance of connective tissue strongly suggests that the site of action of vitamin P on the capillary wall is the pericapillary layer.

The full significance of vitamin P deficiency on the body economy is as yet unknown, as very little investigative work has been carried out in this direction. Duran-Reynals (20) has reviewed abundant evidence which indicates that susceptibility to infection varies directly as the degree of permeability of connective tissue. In experimental arteriosclerosis, Duff (21) has shown that damage to vascular ground substance precedes deposition of lipoids. Gorev and Smirnova-Zamkova (5) maintain that in hypertension and arteriosclerosis, there are degenerative changes in the entire system of ground substance. In view of the role of vitamin P in maintaining the stability of ground substance, it is possible that the long term sequel of vitamin P deficiency is an increased susceptibility to infectious and degenerative processes.

#### SUMMARY

Rutin and esculin, two types of vitamin P, inhibited the spread of intradermally injected hyaluronidase, azoserum and saline. The results are believed to be due to a non-specific increase in the resistance of the connective tissue ground substance. In vitamin P deficiency, induced by feeding turnip antivitamin-P factor, the permeability of connective tissue was increased. This was partially reversed by the administration of esculin, but not by ascorbic acid.

In addition to demonstrating a wider site of action of vitamin P than hitherto recognized, the experimental findings add further evidence in favor of the existence of this vitamin. It is suggested that vitamin P regulates capillary resistance and permeability by an action on the pericapillary sheath. It is suggested that the weakening of ground substance by vitamin P deficiency might predispose to the development of degenerative and infectious diseases.

The author wishes to express his thanks to Professor H. E. Hoff for his constructive criticisms in the preparation of this manuscript and to Mr. J. Richard for his invaluable technical assistance.

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# POTENTIATION OF TWITCH TENSION AND PROLONGATION OF ACTION POTENTIAL INDUCED BY REDUCTION OF TEMPERATURE IN RAT AND FROG MUSCLE<sup>1</sup>

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DURING the past four years it has been observed that the rising time and the peak tension of the twitch of the *in situ* rat triceps surae were slightly greater in experiments carried out in the winter than in those done in the summer. The room temperature was about 26° C. in the winter and about 31° C. in the summer. An effect of temperature changes on the modification of twitch response in the leg muscle suggested itself because it seemed likely that the temperature of anaesthetized animals may decrease more in the cooler room. No report on the effects of temperature changes on twitch tension of *in situ* mammalian muscle was found in the literature. However, it was found that an observation (1) on the effects of cooling on the isolated nerve-diaphragm preparation of the rat had been made. In the fatigued preparation cooling increased the rising time, tension and action potential duration in response to single indirect shocks. The increased duration of action potential produced by cooling skeletal muscle (2) is of interest in the present report because it has recently been shown (3) that the potentiation of tension and the increase of rising time of the twitch in rats given intraperitoneal injection of KCl are accompanied by an increase in duration of the action potential. The present study was begun in order to determine the changes in mechanical and electrical response of *in situ* rat muscle induced by lowering the temperature approximately 10° C. below normal. Discrepancies between our findings and the observations (4, 5) on isolated frog muscle suggested that experiments on frog muscle with intact circulation should be carried out. A preliminary report on this study was made before the American Physiological Society at the meeting in Minneapolis in September, 1948.

## METHODS

Male rats, of the Anheuser-Busch strain, weighing approximately 275 gm. were anesthetized with 300 to 350 mg. of sodium barbital per kg. The experiments with the control group of rats were done at room temperature (31° C.). Sixteen rats were placed in a cold room (10° C.) until the rectal temperature was reduced to about 20° C. Preparation for recording was made after removal from the cold room in 8 animals and the preliminary portion of preparation was made before removal in 5 animals. Three rats were prepared and stimulated in the cold room. The muscle of 3 rats was cooled by placing the leg in a cooling chamber. The triceps surae was pre-

Received for publication February 21, 1949.

<sup>1</sup> Aided by a grant from the U. S. Public Health Service.

pared for stimulation and recording as previously described (6). Action potentials were recorded from the gastrocnemius muscle with a cathode ray oscillograph, one lead electrode being placed in the belly and the other in the tendon of the muscle. The myograms were made with an isometric lever. Determinations of rat plasma K were made with a flame photometer designed by Dr. Theodore Weichselbaum and Dr. P. L. Varney.

The gastrocnemius muscle of the frog was cooled by placing the leg in Ringer's solution maintained at the desired temperature with a water bath, the hind legs being immobilized by section of the spinal cord at a level which did not impair breathing.

Some definitions and qualifications of terms employed in the presentation of the results and the discussion are given for purposes of clarification. The expression, membrane 'breakdown,' is used for convenience in reference to changes in the fiber membrane during the passage of the excitation wave. The twitch is the single response of all component muscle fibers to a single indirect shock, the shock strength being 3 to 4 times maximal. The term potentiation designates an increase of twitch

TABLE I. AVERAGE VALUES FOR THE PEAK TENSION AND TIME COURSE OF THE TWITCH IN THE IN SITU TRICEPS SURAE OF THE RAT WHICH SHOW THE MODIFICATIONS INDUCED BY REFRIGERATION OF THE ANIMAL AT 10° C.

NO. OF RATS	GROUP NO.	ROOM TEMPERATURE	RECTAL TEMPERATURE	TWITCH TENSION	RISING TIME	TIME OF HALF FALL	TIME OF HALF FALL/RISING TIME
8	1	31	26.1 ± 0.52 <sup>1</sup>	434 ± 9.6	35.6 ± 2.37	38.1 ± 4.14	1.06 ± 0.04
5	2	26	22.0 ± 0.35	523 ± 6.8	74.2 ± 1.02	92.8 ± 3.7	1.24 ± 0.03
4	3 <sup>2</sup>	31	36.4 ± 0.34	270 ± 10.4	14.2 ± 1.05	21.5 ± 1.19	1.57 ± 0.04

<sup>1</sup> Standard error of the mean =  $\sqrt{\frac{\sum d^2}{n(n - 1)}}$ ,   <sup>2</sup> Control group given no refrigeration.

tension resulting from increased contraction of the component fibers. Tension is expressed in gm./gm. of fresh muscle; the measurements as cited in this paper refer to developed tension. The resting tension was usually approximately 75 gm./gm. of muscle. The rising time was measured from the beginning of the upward deflection of the record to the peak. The time of half fall was measured from the peak of the tension curve to the point where this curve has returned half way to the initial resting tension.

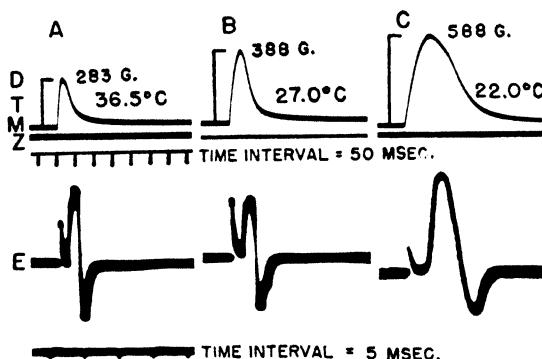
## RESULTS

*Observations on the Rat.* The rats in group 1 of table I were removed from the refrigerator when the rectal temperature had fallen to about 21° C. and prepared for stimulation at a room temperature approximately 31° C.; these experiments were carried out in August. The records were obtained by the time the rectal temperature had risen to 25° C. to 29° C. A comparison of average values in this group of animals with those obtained in control rats (group 3) shows a 61 per cent increase of peak tension, a marked increase (151%) of rising time and a smaller increase (77%) in time of half fall in the twitch response of the cooled animals. The rats in group 2 were par-

tially prepared for stimulation before removal from the refrigerator and the records were obtained by the time the rectal temperature had increased to about  $22^{\circ}$  C. at a room temperature approximately  $26^{\circ}$  C.; these experiments were carried out in November. Under these conditions 94 per cent, 423 per cent and 331 per cent increases, respectively, of tension, rising time and time of half fall were induced. The rising phase of the twitch was prolonged more than the falling phase, this difference being more pronounced with moderate cooling (cf. group 1 with group 2 in table 1 and B with C in fig. 1). The myogram in figure 1B shows the least potentiation of twitch tension obtained in group 1 and the myogram in figure 1C shows the greatest potentiation obtained in group 2 of table 1. These myograms illustrate the progressive slowing of the twitch response by cooling.

The marked difference in duration of twitch response in groups 1 and 2 of table 1 suggested that the difference in muscle temperature may be greater than that indi-

FIG. 1. EFFECTS OF REFRIGERATION of the rat on the triceps surae mechanical response and gastrocnemius action potential, the muscles being stimulated indirectly with single shocks. D. Developed tension expressed in gm./gm. of fresh muscle. The resting tension was approximately equal for the records shown in this fig. T: rectal temperature at the time the records were made; M: myograms; Z: zero tension; E: action potentials; A: records made 2 hours after giving sodium barbital; B: records made after 1 hour of refrigeration at  $10^{\circ}$ C.; C: records made after 2.5 hours of refrigeration at  $10^{\circ}$ C. The action potential record in C was retouched.



cated by the rectal temperature. Because the animals in group 1 were prepared for stimulation in a warm room ( $31^{\circ}$  C.) it seemed likely that the muscle temperature may have been much higher than the rectal temperature at the time of recording. In order to follow more closely the temperature changes in the muscle the stimulating and recording apparatus was placed in the refrigerator. In a warm room a series of 3 rats was prepared for stimulation and a small thermometer was placed beside the contralateral gastrocnemius muscle through a small incision near the heel. The animals were then placed in the refrigerator for periodic stimulation and recording. The results of one of these experiments are shown in figure 2. The peak twitch tension increased sharply as the muscle temperature decreased from  $37^{\circ}$  to  $30^{\circ}$  C., and less rapidly as the temperature fell from  $30^{\circ}$  C. to  $24^{\circ}$  C. The decline in rate of potentiation of tension in the lower range of temperature was accompanied by a marked increase in duration of the twitch response. The sharp increase of tension observed in the higher range of temperature was accompanied by a less marked increase in duration of the twitch response. The effects obtained by cooling the muscle gradu-

ally disappeared as the muscle was allowed to return to normal body temperature. At 37° C. the twitch duration was normal and peak twitch tension was slightly above normal. The results obtained during the progress of refrigeration (fig. 2) indicate that the muscle temperature was about 30° C. in group 1 of table 1 and about 24° C. in group 2.

The effects of cooling on the action potential are of particular interest in the experiments done during the progress of refrigeration because it was possible to observe the changes at different temperatures with the recording electrodes in the same position. The duration of the action potential increased slowly at first and then more abruptly as the muscle temperature fell below 32° C. (fig. 2). The height of the action potential increased initially but it decreased toward the normal height as the muscle temperature fell below 29° C. The increase in duration of the action potential is very similar to the increase observed during the progress of twitch potentiation induced by intraperitoneal injection of KCl in the rat (3). It should be noted that the height and duration of the action potential were normal when the muscle was allowed to return to normal body temperature.

The muscle was cooled in 3 rats by placing the leg in a cool chamber (17° C.-20° C.) for about 20 minutes. This treatment brought about a  $37 \pm 4$  per cent increase of peak tension, a  $172 \pm 7$  per cent increase in rising time, a  $308 \pm 25$  per cent increase in time of half fall and a  $253 \pm 34$  per cent increase in duration of the action potential. Records from one of these experiments are shown in figure 3. Although the action potential height was lower in 2 of the 3 experiments at the time maximum potentiation was attained, the height increased initially in all 3 experiments.

*Effects of Local Cooling in the Frog.* The results obtained by cooling the *in situ* gastrocnemius in 3 summer frogs with intact circulation and normal respiration were similar to those obtained in the rat by moderate refrigeration. The average high temperature of the Ringer's solution bath in which the leg was immersed was 27.5° C.; the average low temperature was 14.5° C. Change of the temperature from the high to the low level produced a  $40 \pm 9$  per cent increase of peak tension, a  $200 \pm 8$  per cent increase of rising time and a  $154 \pm 30$  per cent increase in time of half fall in the twitch response. The records in figure 4 show typical effects of the extreme temperatures on mechanical and electrical responses. Records taken at intermediate temperatures showed intermediate modifications of twitch tension and time course and of action potential duration.

Similar cooling of isolated summer and winter frog gastrocnemii produced varied effects on twitch tension. In 5 experiments the peak tension was decreased and in one it was increased by reduction of the temperature of the bath. The slowing of the twitch was about the same as that observed in the cooled intact frog muscle. Similar results were obtained in *in situ* muscle of winter frogs; cooling induced a slight decrease of gastrocnemius twitch tension in 3 and a small increase in 1 of 4 experiments.

Since the plasma K is markedly increased in rats showing potentiation of twitch tension after KCl treatment (3) the possibility that a shift of K to the extracellular fluid may be responsible for the potentiation of twitch tension in cooled muscle was explored. The average values for analyses of plasma K were 4.85 mM per liter in 5 rats cooled to 20° C. and 3.99 mM per liter in 3 control animals.

## DISCUSSION

It seems unlikely that the 24 per cent increase of plasma K found in rats cooled to 20° C. plays a major rôle in bringing about the 94 per cent increase of twitch tension in these animals, because a previous study (3) has shown that a 200 per cent in-

FIG. 2. DIAGRAM SHOWING CHANGES IN RESPONSE of the rat triceps surae to single indirect shocks as the muscle temperature was reduced from 37°C. to 24.5°C. and then increased to 37°C. The muscle was cooled by placing the anesthetized animal in a cold room (10°C.). The intervals between the recordings were 10 minutes in all cases except the first, which was 5 minutes, and the last, which was 75 minutes. The peak tension of the twitch is expressed as gm./gm. of fresh muscle. The recorded action potential height is given in mm.

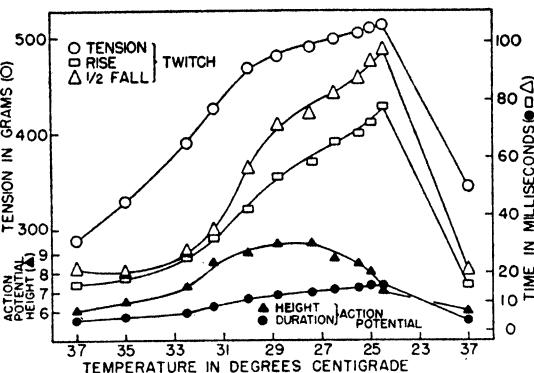


FIG. 3. EFFECTS OF COOLING THE LEG in a chamber on the response of the rat triceps surae to single indirect shocks. D: developed tension expressed in gm./gm. of fresh muscle; T: rectal temperature before local cooling; A: rectal temperature after local cooling. M: myograms; Z: zero tension; E: action potentials.

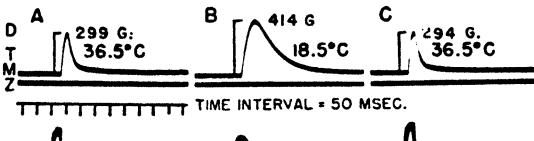
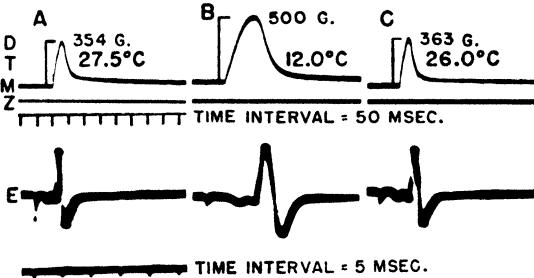


FIG. 4. EFFECTS OF COOLING THE LEG in a Ringer's solution bath on the response of the frog gastrocnemius to single indirect shocks. D: developed tension expressed in gm./gm. of fresh muscle; T: temperature of the bath; M: myograms; Z: zero tension; E: action potentials.



crease of plasma K concentration resulting from intraperitoneal injection of KCl produces only a 30 per cent increase of twitch tension. It should be noted, however, that both K treatment and cooling produce prolongation of the action potential and an increase of the ratio of rising time to falling time in the potentiated response of rat muscle to single indirect shocks.

Findings on the effect of temperature on the response of isolated frog muscle to single shocks are contradictory. For example, Gad and Heymans (4) reported that

a reduction of temperature from 30° C. to 19° C. induced a decrease of tension development, while Bernstein (5) found that similar reduction of temperature may either increase or decrease tension development in response to single shocks. Other reports in the literature agree with the findings of Gad and Heymans. The observation in this study that reduction of temperature from 27.5° C. to 14.5° C. increased twitch tension of *in situ* muscle in summer frogs, but usually decreased twitch tension of *in situ* muscle in winter frogs and of isolated muscle from summer and winter frogs, suggests that muscle response to temperature changes depends upon the nutritional state of the muscle. The responsible factors are not yet recognized. The potentiation of twitch tension obtained by local cooling in the rat and in the frog rule out the possibility that the effects of cooling on muscle response are due to metabolic changes induced by refrigeration of the whole animal.

The suggestion in connection with studies on the effects of KCl treatment, that increased duration of action potential as recorded from a multifibered muscle may be due in part to increased duration of membrane 'breakdown,' is supported by observations on the effects of cooling. Sanderson (2) found that action potential duration is increased and that conduction rate is reduced in the frog sartorius muscle by cooling. On the basis of his observations he suggested that cooling increases the duration of potential disturbance at a given point during the passage of an excitation wave. From the data recently reported (7) on the effect of temperature changes on the conduction rate and spike duration in single motor nerve fibers of the toad it is possible to calculate the change in length of the excitation wave during the passage of an impulse. The wave length was approximately doubled by reduction of the temperature from 25° C. to 5° C. The duration of the action potential was increased about 5 times by the same change of temperature. In so far as the findings for nerve fibers are applicable to muscle fibers these data support the view that duration of membrane 'breakdown' is greater in a cool than in a warm muscle fiber during the passage of an excitation wave. Whether there is a causal relation between increased duration of membrane 'breakdown' and increased contraction of muscle fibers, capable of responding with such increase of tension, cannot be decided with certainty. The view that such a causal relation exists is consistent with the findings on the rat and on the *in situ* summer frog muscle, and can be reconciled with the findings on the winter frog muscle and on isolated frog muscle by making the assumption that such muscles are incapable of responding with an increased tension.

#### SUMMARY

Observations were made on the modification of twitch response induced by cooling the rat triceps surae *in situ* and the frog gastrocnemius both *in situ* and isolated. The rat muscles were cooled by refrigeration of the whole animal or by placing the leg in a cooling chamber. The frog muscles were cooled by placing the leg or the isolated muscle in cool Ringer's solution.

Cooling the rat triceps surae from 37° C. to 24° C. by refrigeration increased twitch tension about 80 to 90 per cent and increased the duration of the action potential about 300 per cent. The time course of the twitch was increased about 350 per cent. The rising phase of the twitch was prolonged more than the falling phase, this

difference being more pronounced in the case of moderate cooling. Reduction of the temperature of the bathing fluid from 27.5° C. to 14.5° C. induced changes in the twitch response and action potential of the *in situ* gastrocnemius muscle of summer frogs similar to those observed in rat muscle. The same change of temperature usually produced a decrease of twitch tension of *in situ* muscle of winter frogs and of isolated frog muscle.

Although the effect of cooling in the rat apparently is not due to a change in concentration of plasma K, moderate cooling and K treatment induce similar changes in time course and tension of the twitch and in duration of the action potential. Support is offered for the view that the duration of membrane 'breakdown' produced by the passage of an excitation wave along the muscle fiber is prolonged by cooling. A causal relation between increased duration of membrane 'breakdown' and increased contraction of muscle fibers is suggested.

The author is indebted to Dr. W. A. Quebedeaux for technical assistance.

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# OXYGEN CONSUMPTION AND COOLING RATES IN IMMERSION HYPOTHERMIA IN THE DOG<sup>1</sup>

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MANY observations have been made on the effect of reduced body temperature on oxygen consumption. Overall conclusions are made difficult, however, by the wide range of experiment designs. Whether anesthesia was used, if so, depth and type, method of inducing hypothermia, duration of exposure, and species variation are but a few of the many factors tending to preclude generalizations. Too, variation in shivering response within a given set of experiments must be considered.

While the rate of cooling of the intact organism has received considerably less critical analysis, it likewise is subject to the same variables mentioned above. In an effort to derive a clear picture of the response of the hypothermic dog a series of experiments was designed wherein the procedure was constant. From these experiments it is apparent that shivering, which is in large measure an individual characteristic (at least under anesthesia), has a profound influence on oxygen consumption and rate of cooling. Only at the lowest extremes of body temperature, below which shivering is never seen, is it possible to predict accurately the metabolic response of a given animal.

## METHODS

Fifteen experiments were conducted on 7 unselected but generally healthy mongrel dogs. On two occasions, separated by about 10 days, each dog was cooled to a rectal temperature of approximately 20°C. and rewarmed. In one case the dog was cooled a third time, using a longer lasting anesthetic.

After being fasted 24 hours the undepilated<sup>2</sup> dog was anesthetized with sodium pentothal<sup>3</sup> per venam and secured in a supine position to a dog board. An endotracheal tube and rectal thermocouple were inserted and duplicate O<sub>2</sub> consumption determinations were made using a Sanborn respirometer. The preparation was then lowered into a tub of iced water (2°-4°C.) in a position about 10° from the horizontal. Immersion was complete except for the head, neck and ventral portion of the chest. The O<sub>2</sub> consumption was recorded continuously and the rectal and bath temperatures every 24 seconds.<sup>4</sup> Pulse rates and shivering were observed and noted by a recorder. Additional pentothal was supplied as indicated to keep the anesthesia just adequate. The average total pentothal dose was 32 mg/kg. body weight, given in 5 per cent solution. In only one instance was more pentothal required at a rectal temperature below 35°C., the cold effect apparently having sufficiently augmented the anesthesia by this time to keep the dog quiet.

Received for publication January 25, 1949.

<sup>1</sup> This work was carried out under Contract W33-038-ac-14757 with the Aeromedical Laboratory, Air Materiel Command, Wright-Patterson Air Force Base, Ohio.

<sup>2</sup> A series of trials by Haterius (3) and an observation by Speelman (2) failed to reveal any influence of depilation on rate of cooling in the dog.

<sup>3</sup> Kindly donated by Abbott Laboratories, Inc.

<sup>4</sup> 'Speedomax' recorder, Leeds & Northrup Co., Philadelphia.

At a rectal temperature of 20°C. the preparation was removed from the cold bath and rewarming measures at once instituted. These measures took the form of spraying with water of 40° to 42°C. for about 5 minutes following which the dog was left in room air of 25° to 28°C. to complete the rewarming, or immersion in a tub of water at 40°C. throughout the temperature ascent, or combinations of immersion in warm water and exposure to room air. With the return of consciousness and motor activity the measurements were terminated.

### RESULTS

*Oxygen Consumption during Cooling.* The O<sub>2</sub> consumption before immersion and at rectal temperatures of 35°, 30°, 25° and 20°C. are presented in table 1.

TABLE I. OXYGEN CONSUMPTION OF DOGS, CC./KG./MIN. (STPD), DURING COOLING BY IMMERSION IN COLD WATER

DOG	EXPER.	WT. KG.	38-39°	35°	30°	25°	20°
1	A	9.4	6.9	8.2	10.3	6.0	1.1
	B	7.9	6.3	4.8	4.2	4.6	1.0
2	A	13.6	6.3	4.2	5.7	5.1	1.6
	B	14.0	7.3	5.9	4.7	4.8	1.9
3	A	15.6	5.0	5.0	4.9	2.5	2.2
	B	14.0	6.0	4.4	4.8	3.5	1.0
4	A	14.0	6.2	6.0	11.5	8.4	1.9
	B	12.8	5.8	12.5	18.0	8.0	0.9
5	A	20.4	5.6	4.0	2.3	2.2	0.9
	B	20.8	6.4	3.6	2.8	2.3	1.1
6	A	15.6	4.9	4.0	10.2	12.4	1.1
	B	15.1	5.0	4.7	18.3	12.0	1.0
	C <sup>1</sup>	15.1	6.6	5.0	4.8	1.5	—
7	A	10.7	8.4	7.4	7.2	4.8	1.0
	B	9.5	7.1	5.0	4.0	3.0	0.7

<sup>1</sup> Deeply anesthetized with Na-Amytal.

Individual graphs were plotted for each experiment and were seen to be separable into 4 general patterns. A typical representative of each of these patterns is plotted in figure 1, together with the numbers of experiments of which each curve is typical. *Experiment 15*, in which sodium amytal anesthetic was used in place of sodium pentothal, is not included in this group.

In the 3 experiments wherein a very high peak in O<sub>2</sub> consumption was noted the dogs exhibited violent shivering. The 2 dogs showing the moderate rise in metabolism likewise showed a moderate shivering response, while the 7 showing a very late, small rise shivered only minimally. It is of interest that, in the latter case, the shivering began approximately 25 minutes after pentothal anesthetization, which is in accord with the usual duration of this anesthetic in the absence of cold. But

seemingly at the temperature of emergence from the barbiturate depression the organism is not capable of great shivering.

It would appear from our data that the  $O_2$  consumption response of the dog is largely an individual one inasmuch as 5 of the 7 dogs showed the same category of response both times they were cooled. The only pattern which was not repeated by at least 1 dog was that of curve B, figure 1, wherein both dogs which exhibited this response on the first cooling showed an altered pattern the second time, one falling into the pattern of curve A and the other of curve C.

The effect of deep narcosis on  $O_2$  consumption during hypothermia was investigated by cooling for a third time the dog which twice under sodium pentothal showed the very high peak of curve A, figure 1. In this instance sodium amyral (50 mg/kg.) was the anesthetic and in this experiment (no. 15, table 1) no shivering was evidenced

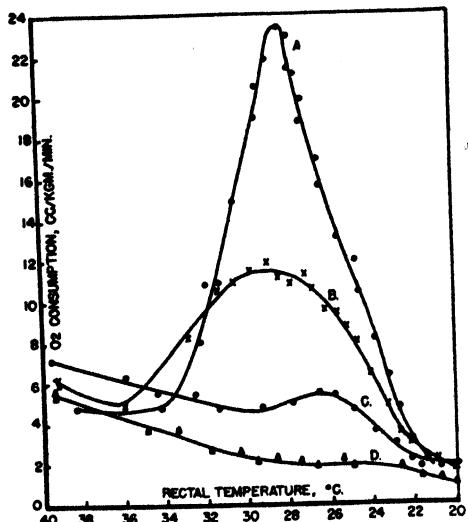


Fig. 1. TYPICAL EXAMPLES of the 4 types of oxygen consumption exhibited by hypothermic dogs. The no. of experiments in which each pattern was shown was as follows: A-3, B-2, C-7, D-2.

and the metabolism fell linearly to 24°C. rectal temperature, at which point respiration ceased. Resuscitation was instituted and successful rewarming effected. Three months later this animal was again cooled and rewarmed under pentothal anesthesia twice more with essentially the same response as experiments 6 and 13 of table 1.

*Oxygen Consumption during Rewarming.* In the first 7 experiments wherein rewarming was effected by spraying with warm water for about 5 minutes followed by exposure to room air of 25° to 28°C. shivering began between 24° and 28°C. rectal temperature. The  $O_2$  consumption showed an almost simultaneous sharp rise and in all cases but one greatly exceeded the pre-immersion control level. The one exception occurred in the dog which did not shiver during cooling; and even in this case it is likely that, had the measurements not been terminated due to returning consciousness at 29.5°C., the control level would ultimately have been surpassed. Curve A of figure 2 is a typical  $O_2$  consumption pattern of a dog rewarmed in air.

Fig. 2. OXYGEN CONSUMPTION PATTERN during rewarming in the hypothermic dog. Curve A: dog rewarmed in air ( $25\text{--}28^{\circ}\text{C}$ .) following 5 minutes of spraying with  $40^{\circ}\text{C}$ . water; Curve B: same as A except that at rectal temperature  $27.3^{\circ}\text{C}$ . dog was immersed in warm water ( $40\text{--}42^{\circ}\text{C}$ .) for duration of rewarming; Curve C: dog immersed in water at rectal temperature  $20^{\circ}\text{C}$ . and removed to room air at rectal temperature  $30^{\circ}\text{C}$ .

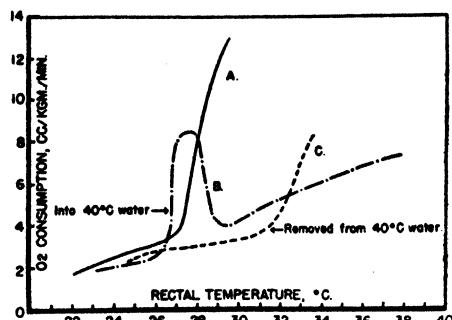


TABLE 2. RATE OF COOLING OF DOGS IMMERSED IN AN ICED WATER BATH ( $2^{\circ}\text{--}5^{\circ}\text{C}$ ).  
Anesthetic: Pentothal Sodium

DOG	EXPER.	WT. <i>kg.</i>	TOTAL COOLING TIME <i>min.</i>	BEGINNING RECTAL TEMP. <i>C°</i>	LOWEST RECTAL TEMP. <i>C°</i>	MEAN COOLING RATE <i>°C/min.</i>	TYPE OF $\text{O}_2$ CONSUMPTION PATTERN
1	A	9.5	68	38.8	21.2	0.259	Moderate rise
	B	7.9	59	38.9	20.0	0.320	Small, late rise
2	A	13.6	75	39.2	20.0	0.256	Small, late rise
	B	14.0	54	39.9	20.3	0.363	Small, late rise
3	A	15.6	52	38.5	19.4	0.367	Small, late rise
	B	14.0	67	40.0	21.8	0.272	Small, late rise
4	A	14.0	127	39.7	19.6	0.158	Moderate rise
	B	12.8	115	39.8	21.8	0.156	Very great rise
5	A	20.4	92	40.1	20.1	0.218	No rise
	B	20.8	80	39.5	21.2	0.229	No rise
6	A	15.6	164	39.7	20.0	0.120	Very great rise
	B	15.1	140	38.8	21.7	0.122	Very great rise
	C <sup>1</sup>	14.4	82	38.9	24.0	0.182	No rise
7	A	10.7	70	39.9	20.6	0.276	Small, late rise
	B	9.5	53	38.9	21.0	0.338	Small, late rise
168 <sup>a</sup>		11.0	100	39.0	20.3	0.187	Dead
335 <sup>b</sup>		9.4	101	40.0	20.0	0.198	Dead
336 <sup>b</sup>		6.0	121	39.0	20.0	0.157	Dead

<sup>1</sup> Deeply anesthetized with Na-Amytal.

<sup>a</sup> Freshly killed dogs.

In the second half of the experiments it was found that the pronounced shivering and concurrent sharp  $\text{O}_2$  consumption increase on rewarming could be controlled by immersion in a bath of warm water ( $40^{\circ}\text{--}42^{\circ}\text{C}$ .). The shivering, regardless of mag-

nitude, could be stopped almost immediately and the  $O_2$  consumption would, within a short time, fall back to a line connecting the pre-immersion control level with that of  $20^\circ C$ . A typical representative of this pattern is shown in curve B of figure 2.

At no time during the course of these experiments was shivering observed while the dog was immersed in warm water. However, removal from warm water to air was invariably, and often within seconds, followed by the initiation of shivering. In one instance the animal was kept in warm water to a rectal temperature of  $30^\circ C$ . with no evidence of shivering. Shortly after removal shivering began and the  $O_2$  consumption curve took a sharp upward turn. This response is plotted as curve C of figure 2.

*Rates of Cooling.* Through the use of the temperature recorder the opportunity was presented for the determination of precise rates of cooling. These data are presented in table 2, from which it may be seen that the mean cooling rates vary considerably between dogs as well as within the same dog on different days. Four of the 7 dogs showed a faster cooling rate on the second exposure while 1 showed a

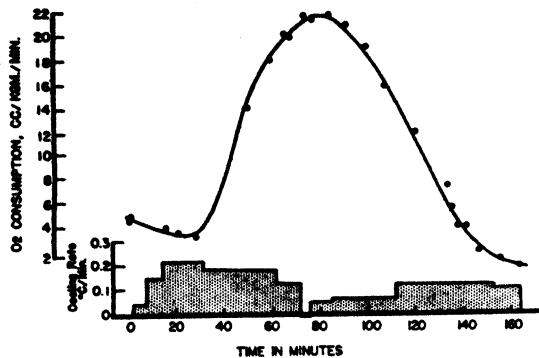


Fig. 3. CHANGES IN RATE of cooling and  $O_2$  consumption of a dog immersed in an iced bath. This dog showed great shivering.

slower rate and in 2 there was effectively no change. The fact that the mean cooling rate on the second exposure was 8 per cent faster than on the first is not thought to be significant.

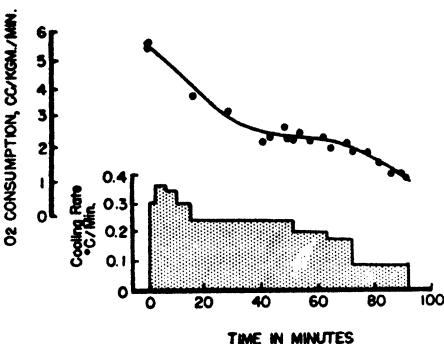
If the mean cooling rates be separated in accordance with the 4 categories of  $O_2$  consumption discussed previously a good correlation is obtained. In the 3 experiments wherein the  $O_2$  consumption reached a high peak, the mean cooling rate was  $0.133^\circ C/min.$ ; in the two in which the rise was moderate,  $0.208^\circ C/min.$ ; in the 7 showing the late, slight rise,  $0.313^\circ C/min.$ ; and in the case of the dog which showed a progressive declination both times,  $0.223^\circ C/min.$  Only in the latter case is the value not in complete accord with the amount of heat production by the animal as evidenced by his  $O_2$  consumption. In the 1 experiment wherein the dog that had shivered violently during both coolings under pentothal was cooled again under sodium amytal with no evidence of shivering the cooling rate increased 54 per cent.

Data from 3 previous experiments involving freshly killed dogs are added to table 2. In these dogs it is seen that the mean cooling rate to  $20^\circ C.$  does not differ materially from that of the deeply anesthetized dog and is slower than all but 4 of the 14 observations on lightly anesthetized dogs.

The rate of cooling at any instant is closely associated with the metabolic activity. Evidence for this is seen in figure 3 in which the cooling rate is plotted against the  $O_2$  consumption in a dog which exhibited a great deal of shivering. Of primary interest in this graph is the fact that for a period of 4 minutes during the peak of the  $O_2$  consumption and shivering, this dog was able to effect a reversal of the cooling trend and actually rewarm slightly even in the face of the water bath temperature of 3°C.

In the absence of shivering and metabolic increase during cooling the rate of cooling becomes high soon after immersion and progressively decreases as the rectal temperature of the animal approaches that of the environment. This relationship is pictured in figure 4.

Fig. 4. CHANGES IN RATE of cooling and  $O_2$  consumption of a dog immersed in an iced bath. This dog did not visually shiver.



#### DISCUSSION

Although the use of anesthetized animals for observations of this type has been questioned from a physiological point of view (1) it proved desirable from many standpoints to induce a degree of light narcosis during the initial stages of cooling. Spealman (2) has shown that hypothermia in the unanesthetized dog is difficult to obtain by immersion in cold water. Cold itself, after sufficiently low body temperatures have been reached, acts as a narcotic which as pointed out by Haterius and Maison (3) is superimposed upon a long-acting anesthetic. For this reason pentothal sodium, which under normal conditions has a relatively short duration of action, was used on the theory that the cold would ultimately replace, rather than augment, the barbiturate effect. No direct proof for this supposition can now be offered, but this problem is being investigated.

The use of rectal temperature as a criterion of body temperature in immersion experiments has also been questioned (4). There can be no doubt that in this type of experiment temperatures of different organs will vary appreciably, especially at the lower ranges. However, if care is taken in positioning the rectal thermocouple its temperature does not differ widely from that of other deep areas of the body above a temperature of 22°C. A fuller discussion of these temperature relationships will be included elsewhere.

Grosse-Brockhoff and Schoedel (5) and Woodruff (6) have reported observations of  $O_2$  consumption in the hypothermic dog. The former authors found a maximum

increase of 6 times, usually 3 to 4 times, in the early phase of the cooling of lightly anesthetized dogs. They reported the peak to be passed at  $33^{\circ}$  to  $30^{\circ}\text{C}.$ , a value somewhat higher than in our experiments. Likewise they reported that the metabolic level had always sunk below the pre-immersion value before the rectal temperature reached  $25^{\circ}\text{C}.$  In the 5 experiments of our series wherein the shivering was considerable the  $\text{O}_2$  consumption did not sink below the pre-immersion control until a somewhat lower temperature was reached.

Neither of the above groups cooled their animals to a temperature as low as  $20^{\circ}\text{C}.$  nor did they report classes of response during cooling. The fact that only below about  $23^{\circ}\text{C}.$  rectal temperature, below which shivering is no longer a factor, is it possible to predict with any degree of accuracy the  $\text{O}_2$  consumption of a given dog is believed to be an important aspect of our findings. It is entirely conceivable that this considerable difference in response of dogs is related to their inherent susceptibility to barbiturate anesthesia. It is not, however, correlated with quantity of pentothal. It is probable that, with enough animals, the 4 classes of response described above would give way to a complete spectrum of response varying from the very high peak in the dogs that shiver freely to the total absence of any peak in those dogs which fail to mobilize their defenses to the point of shivering. Moreover, in the absence of any depressant effects of anesthesia, it is likely that the predominant effect would be nearer the top of this spectrum than at the end wherein no increase was apparent.

Many authors (7-12) have reported an initial stimulation followed by depression in the metabolic level of unanesthetized rats subjected to cold air or cold water. The peak does not appear to be as high, relative to pre-cooling controls, as in the case of dogs. This is doubtless related to the fact that the shivering ability of the rat is generally less than that of the dog.

In the case of human patients, the observations have largely been made after prolonged exposures to cold. Smith and Fay (13) reported a BMR reduction of 6 per cent to 25 per cent, but Dill and Forbes (14) found a decrease in only 6 of their 28 observations made at temperatures of  $25.5^{\circ}$  to  $38^{\circ}\text{C}.$  Talbott (15) believes that the metabolism may well remain above the based level throughout the period of hypothermia, while Herrmann (16) and Geiger (17) hold that with prolonged hypothermia the BMR will be reduced, possibly showing a short period of stimulation early in the cooling.

The sudden and dramatic cessation of shivering upon immersion in warm water during the rewarming phase of these experiments was striking. The onset of shivering following removal from warm water to room air at rectal temperatures between  $24^{\circ}$  and  $30^{\circ}\text{C}.$  was almost as sudden. Uprus, Gaylor and Carmichael (18), using human subjects, observed a rapid shivering response to hot and cold baths, but, from their measurements of concurrent rectal temperatures, concluded the effect was of central origin and always correlated with a rise or fall of blood temperature. Jung, Doupe and Arnold (19) take exception to this view and hold that peripheral stimuli play a major rôle in this effect. Hemingway (20), using diathermy treatment on

dogs, found results which seem to substantiate Uprus *et al.*, but, from the suddenness of the response, our results favor the theory of peripheral origin.

Our data on rates of cooling do not appear to confirm a close relationship to body size. It is possible, however, that this effect was masked by the large variability in shivering. It is significant that in 10 of our observations the mean cooling rate was faster than is the case with a dead dog and only in 4 experiments, in which the shivering was great and prolonged, was the live animal able to effect a slower cooling rate. It is thus apparent that the dog is unable to effect sufficient peripheral vasoconstriction to prevent the conduction of a significant amount of heat to the surface even when exposed to extreme cold.

#### SUMMARY

Seven lightly anesthetized dogs were each cooled twice in an iced water bath to a rectal temperature of 20°C. and rewarmed in room air at 25° to 28°C. and/or warm water at 40° to 42°C. The O<sub>2</sub> consumption and rates of temperature change were recorded continuously. One of the dogs which shivered considerably on both coolings was subjected to a third cooling under deep, prolonged anesthesia. In addition, 3 observations of cooling rates of dead dogs are presented.

It is shown that the O<sub>2</sub> consumption varied directly with the shivering response and in the 14 experiments 4 distinct patterns were detectable. It is suggested that variations in shivering response patterns may be a function of susceptibility to barbiturate anesthesia.

Below 23°C. rectal temperature shivering is no longer a factor and the O<sub>2</sub> consumption of all dogs falls to approximately one third that of the pre-cooling control level.

During rewarming all dogs shivered greatly when exposed to room air, beginning between 24° and 28°C. rectal temperature. The shivering could be stopped almost immediately by immersion in warm water. The O<sub>2</sub> consumption pattern followed very closely that of the shivering.

In 4 of the experiments, through profound shivering the dog was able to delay the body cooling such as to make the mean cooling rate slower than that of a dead animal. In the other 10 experiments the cooling rates were all faster than for dead dogs.

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# MAN'S RESPIRATORY RESPONSE DURING AND AFTER ACCLIMATIZATION TO HIGH ALTITUDE<sup>1</sup>

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**I**NCREASED pulmonary ventilation in response to lowered oxygen pressures has long been recognized in man. For a given oxygen pressure, however, this response is not the same for acclimatized and unacclimatized man. In order to analyze the changes which occur during the process of adaptation, various types of experiments have been designed. It is our objective 1) to describe the difference in alveolar air composition between persons acclimatized to various altitudes and persons residing near sea-level who are acutely exposed to similar altitudes and to predict on this basis the ventilatory acclimatization for any altitude, 2) to verify this prediction in experiments on 3 subjects acclimatized to an altitude of 9500 feet for a period of 3 weeks, 3) to describe the respiratory response of men acutely exposed to lower and higher oxygen pressures *after* acclimatization to various altitudes, and 4) to present data which indicate a greater sensitivity of the respiratory system to CO<sub>2</sub> after acclimatization to altitude.

*Difference between Alveolar Air Composition and Ventilation of Acclimatized and Unacclimatized Man at Similar Oxygen Pressures.* The alveolar gas composition of permanent residents as well as sojourners acclimatized to altitude have been collected from reports of various mountain expeditions and are presented in table 1. The alveolar respiratory quotient has been computed in each case from the alveolar gas equation (1, 2).

$$R.Q. = \frac{791 \text{ pCO}_2}{1200(B - 47 - \text{pCO}_2) - \text{pO}_2} \quad \text{Equation 1}$$

The values obtained by Fitzgerald (24) are not indicated in the table since they cover various altitudes from 4000 to 14,000 ft. on permanent residents of Colorado, U. S. A. The averages of her determinations, however, are incorporated in figure 1 and since only CO<sub>2</sub> determination was made, the alveolar oxygen pressure was computed by the alveolar gas equation, assuming a resting R.Q. of .83.

As far as can be ascertained, all samples were collected during rest, and from a forced expiration following a normal expiration. The samples of Helmholtz and Boothby (3), Boothby (2) and Hall and Wilson (4) were taken at the end of inspiration and have been corrected in figure 1 and table 1 to 'end-expiration' samples by adding

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Received for publication February 21, 1949.

<sup>1</sup> Work done under contract with Air Materiel Command, Wright Field, Dayton, Ohio.

<sup>2</sup> The authors are greatly indebted to Dr. L. F. Clark and other staff members of the University of Wyoming Science Camp whose whole hearted cooperation made this study possible.

1 mm. CO<sub>2</sub> and subtracting 2 mm. O<sub>2</sub> (5). It must further be appreciated that posture changes the resting alveolar gas concentrations considerably. It had to be assumed that all observations in the literature were made in the sitting posture.

TABLE I. ALVEOLAR AIR COMPOSITION OF SOJOURNERS AND PERMANENT RESIDENTS AT VARIOUS ALTITUDES AS RECORDED IN THE LITERATURE

NO.	ALTITUDE 1000 FT.	BAR. PRES., MM. HG	NO. SUBJECTS	ALVEOLAR			AUTHORITY
				pCO <sub>2</sub>	pO <sub>2</sub>	R.Q.	
1 <sup>2</sup>	0	760 <sup>1</sup>	16	38.0	106.0	.858	(3)
2	.55	748	22	38.1	100.7	.797	Our data
3 <sup>2</sup>	1.00	733	35	37.7	100.3	.853	(2)
4	4-14		132				(24)
5 <sup>2</sup>	6.2	610	32	33.3	79.5	.866	(4)
6	9.2	543	10	34.0	64.0	.828	(25)
7	10.0	525	8	31.5	61.9	.813	(27)
			3	31.5	63.9	.845	
6	12.5	489	10	30.0	58.0	.850	(25)
7	14.0	446 <sup>1</sup>	3	25.9	51.6	.776	(27)
8	14.2	458		26.8	53.7	.793	(26)
9	14.2	458	4	27.9	52.6	.807	(18)
10	15.4	429	11	28.0	46.9	.820	(28)
	17.5	401	10	25.6	42.3	.761	
	20.1	356	9	21.4	37.7	.761	
11	21.2	331 <sup>1</sup>	2	17.7	42.5	?	(29)
	22.8	310 <sup>1</sup>	2	15.6	37.0	.845	
12	22.7	337	1	17.7	40.7	.860	(30)

<sup>1</sup> Based on Altitude-Pressure Tables, U. S. Stand. Atmos.  
<sup>2</sup> Alveolar values corrected for end-expiratory samples as explained in text.

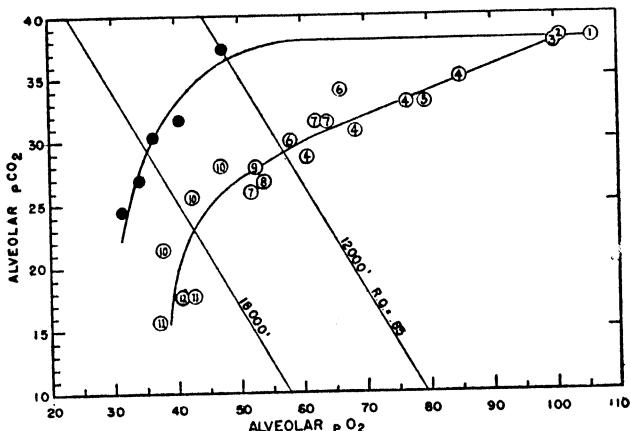
TABLE 2. AVERAGE ALVEOLAR AIR COMPOSITION OF 22 MEN EXPOSED TO VARIOUS SIMULATED ALTITUDES IN A LOW-PRESSURE CHAMBER. SAMPLES WERE TAKEN EVERY 10 MIN. DURING AN EXPOSURE WHICH LASTED ON THE AVERAGE ONE HOUR

ALTITUDE 1000 FT.	BAR. PRES. MM. HG.	NUMBER OF DETERMIN.	ALVEOLAR					
			pCO <sub>2</sub>	S.D.	pO <sub>2</sub>	S.D.	R.Q.	Alv. Vent. Ratio (calculated)
0.55	748	130	38.1	3.5	100.7	6.5	.797	1.00
12.0	483	117	37.4	3.0	47.6	4.7	.830	1.06
16.0	412	48	31.7	4.7	40.7	6.1	.869	1.31
18.0	379	160	30.3	3.8	36.5	4.5	.905	1.43
20.0	349	36	26.9	4.7	34.3	5.1	.918	1.62
22.0	321	23	24.6	3.7	31.3	4.6	.937	1.81

The acute exposures to low oxygen pressures have been carried out by means of a low pressure chamber. Twenty-two young men were exposed, on the average, 1 hour at various simulated altitudes. By far the greatest part of the data was obtained on 8 men who served as regular subjects in these experiments lasting over 3

months. All of these men participated daily either as observers or subjects and were thus well trained and had complete confidence in their work. This absence of all anxiety is a prerequisite in obtaining reliable and typical respiration responses. Alveolar samples were collected approximately every 10 minutes in the sitting position from a forced end-expiration and analyzed by the Haldane method or by the automatic oxygen and carbon-dioxide analyzers described by Rahn *et al.* (5). The alveolar gas concentrations for the acute exposure to various altitudes are listed in table 2 and represent the average for the whole period of exposure.

When the alveolar oxygen and carbon dioxide tensions are plotted on the Fenn  $\text{CO}_2$ - $\text{O}_2$  diagram we obtain 2 distinct curves differentiating acclimatized from unacclimatized men (fig. 1). The iso-altitude line on such a diagram is represented by a



where  $V_a$  equals alveolar ventilation in liters per minute B.T.P.S.;  $X_o$  equals the oxygen consumption in ml. S.T.P.D.; Q, the respiratory quotient, and .864 is a constant. This formula is valid when the inspired air contains no  $\text{CO}_2$ .

In order to compute the relative alveolar ventilation the assumption has to be made that the resting oxygen consumption is unaffected by the altitude. The value obtained at the common origin of these 2 curves in figure 1 at the alveolar  $\text{pCO}_2 = 38.0$  is designated as the *alveolar ventilation ratio* of 1.00. The values taken for calculating the alveolar ventilation curve for the acclimatized men are as follows: the  $\text{CO}_2$  is based upon the average curve presented in figure 1, while for the R.Q. the value of .82 was used which represents the average R.Q. for all determinations in table 1.

For the acute exposure the ventilation ratio was computed only from the alveolar values obtained during the last 10 to 20 minutes of exposure where the R.Q. had returned to an average value of .85 (6). This makes the R.Q., for our purposes, practically identical with those obtained for the acclimatized people. Thus, for both groups the alveolar ventilation becomes only a function of the  $\text{pCO}_2$  and in figure 2 the alveolar ventilation ratio,  $V_a$  R., as well as the alveolar  $\text{pCO}_2$  can be plotted as ordinates against the alveolar  $\text{pO}_2$  and the altitude lines.

Furthermore, the change in serum-bicarbonate of the blood to be expected after acclimatization is completed, can be indicated on the same diagram. If one assumes that the  $\text{pH}$  upon acclimatization returns to normal (7-10), it follows from the Henderson-Hasselbalch equation that the bicarbonate level likewise becomes a function of the alveolar  $\text{pCO}_2$  only.

Figure 2 is particularly helpful in predicting the pathway of the alveolar air composition and alveolar ventilation during the process of acclimatization for any altitude. If we accept these 2 curves, then the process of this adaptation must proceed along a line connecting them. The exact acclimatization pathway, however, is already definitely prescribed by the iso-altitude diagonal as long as the R.Q. remains within normal limits. Thus, for example, the pathways for 5, 10, 15 and 20 thousand feet altitude for an R.Q. of .85 are indicated in figure 2. The acute exposure value after 30 to 60 minutes at these altitudes will lie at the intersection with the unacclimatized curve. The acclimatization pathway will in each case proceed down the altitude diagonal and come to rest at the final intersection with the acclimatized curve. The rate at which this process takes place has been established for the 10,000-ft. altitude and is discussed below. It is of interest to compare the alveolar values obtained by Houston and Riley (8) on 4 men exposed gradually over 4 weeks to increasing altitudes up to 22,000 ft. in a low-pressure chamber. These men never completely acclimatized to any altitude and their alveolar values, described recently in greater detail (38), fall approximately half-way between the 2 curves in figure 2.

The alveolar ventilation curve for acute exposure is of great interest since it indicates no increase in ventilation until the alveolar  $\text{pO}_2$  drops to values below 60 mm. Hg. This corresponds to an altitude of approximately 12,000 ft. or to breathing a mixture of 13 per cent oxygen in nitrogen at sea level and is in general agreement with the recent data of Soley and Shock (11), Dripps and Comroe (12) and Rahn and Otis (6) who measured the *total ventilation* at various oxygen pressures. Furthermore, the alveolar ventilation ratios calculated from the extensive alveolar  $\text{CO}_2$  and  $\text{O}_2$

determinations of Boothby (2) yield values very similar to our own. Thus, it seems quite certain now on the basis of direct measurements as well as calculations based upon the alveolar gas composition that ventilation in man acutely exposed to low oxygen pressures does not increase measurably until the alveolar oxygen pressure drops to about one half of normal (50–60 mm. Hg).

On the other hand, the alveolar air composition of permanent residents or recently acclimatized men indicates a greater than normal (sea level) ventilation. Thus, the hypoxic stimulus is inhibited during at least the first hour in the acutely exposed unacclimatized man. Since below 12,000 ft. altitude this inhibition cannot be due to hyperventilation alkalinity, the only factor that suggests itself is the alka-

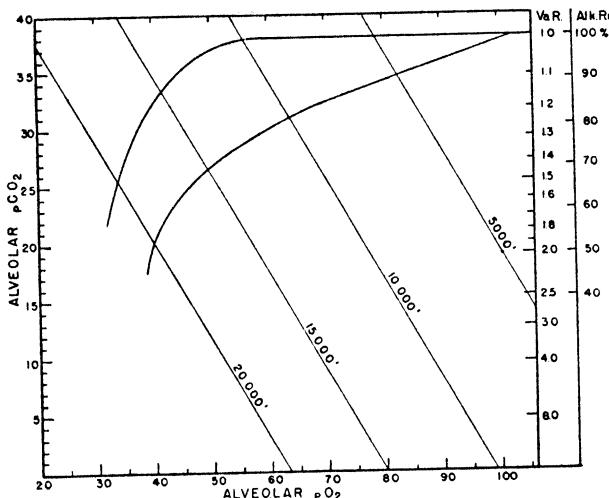


FIG. 2. DIFFERENCE IN ALVEOLAR AIR COMPOSITION and relative alveolar ventilation ratio,  $V_a.R.$ , between unacclimatized man (upper curve) and acclimatized man (lower curve). The calculated change in the alkali reserve, Alk. Res., does not apply to unacclimatized man and applies to the acclimatized man only on the assumption that the arterial  $pH$  has returned to normal. The iso-altitude lines are constructed for an R.Q. = .85.

larity produced by the lowered saturation of the arterial blood. At 12,000 ft. this amounts to about 0.008  $pH$  unit at a  $pO_2$  (alveolar) of 50 mm. Hg and  $pCO_2$  or 38 mm. Hg or an  $HbO_2$  per cent of 86. On the basis of Gray's multiple factor theory this change in  $pH$  would produce an inhibition of about 16 per cent of the alveolar ventilation which is just about balanced by a 20 per cent ventilation increase stimulus shown in the acclimatized man at this altitude (13).

In order to increase the ventilation, therefore, at this altitude one may speculate that compensatory changes in the blood  $pH$  must occur bringing the  $pH$  back to normality before the hypoxic stimulus can effectively exert itself. This is not attained at the end of one hour exposure but appears soon thereafter as will be discussed below.

*Acclimatization of the Respiratory Responses Tested at 9500 Ft.* During the sum-

mer of 1946 three of us took up residence at the University of Wyoming Science Camp located in the Snowy Range at an altitude of 9500 ft. ( $B = 535$ ). Prior to this residence daily determinations were made at Rochester, N. Y., to establish the normal alveolar gas concentration, ventilation volume, respiratory rate and breathholding performance tests. Instrumentation was similar to that used in previous experiments (5). All tests were run between 10 A.M. and 12 M. The subject was comfortably

TABLE 3. ACCLIMATIZATION OF SUBJECTS, A.O. AND R.S. AT 9500 FT. ALTITUDE

DATE	B	ALVEOLAR			VENTI-LA-TION L/MIN BTPS	BREATHS/MIN	BREATH HOLDING BREAKING POINT				EXPOS. TIME	
		pCO <sub>2</sub>	pO <sub>2</sub>	R.Q.			OXIMETER HbO <sub>2</sub> %	pCO <sub>2</sub>	pO <sub>2</sub>	R.Q.		
7-1	744	37.7	101.6	.83	8.58	12.3	49.0	52.9			48	
7-3	754	38.2	103.6	.84	8.78	11.6	50.1	50.2			51	
7-4	755	38.2	104.6	.85	9.08	11.7	48.9	50.4			53	
7-5	753	38.4	104.2	.86	9.24	13.2	51.0	53.6			54	
7-6	749	37.7	103.2	.85	8.76	13.1						
7-3	533	38.9	57.2	.85	89	7.91	12.6	47.4	36.0	71	34	1 hr.
7-10	535	34.0	59.9	.77	88	7.45	11.1	41.8	36.0	0.58	68	33
7-10	536	33.5	59.9	.82		8.63	12.7					18 "
7-11	536	33.1	63.7	.84	91	9.20	12.3	41.8	40.6	0.64	74	29
7-12	536	32.5	61.2	.77		8.48	12.7	40.3	36.7	0.57		32
7-14	536	31.7	62.9	.78	91	8.55	12.9	39.3	39.6	0.59	74	34
7-15	536	30.6	62.3	.74	91	8.67	13.3	38.8	40.6	0.59	75	35
7-16	535	30.6	63.9	.77	90	9.10	13.0	39.0	42.2	0.61	75	30
7-18	535	31.0	63.3	.77	90	8.03	12.7	38.4	43.0	0.60	77	28
7-21	538	30.7	63.8	.75		8.65	12.5	38.4	42.5	0.59		29
7-22	535	31.8	62.9	.76	89	9.35	12.8					
7-28	538	31.9	63.8	.80	88	8.96	10.9	40.0	40.0	0.58		30

Averages on three subjects A.O., R.S. and H.R. before and after acclimatization

Before	751	38.0	103.2	.84		8.68	11.5	49.7	54.7	0.48	58	Roch.
After	536	30.9	63.3	.76	90	8.51	11.5	37.9	44.7	0.61	29	5th to last day

seated and the alveolar air continuously analyzed. When after 10 minutes or longer the alveolar gas concentrations had stabilized, the values were recorded every minute for 5 minutes and averaged. During this period the ventilation volume and breathing rate were automatically recorded on paper tape and averaged. This procedure was followed by 2 breathholding tests (see below). Two days prior to leaving for the mountains similar tests were made in our high-altitude chamber at the end of 1 hour exposure to an altitude of 9500 ft. This acute exposure as can be seen in table 3 showed no change in the alveolar pCO<sub>2</sub> or ventilation as had been predicted.

Rochester, N. Y.

Wyoming—9500 ft.

Wyoming

Twelve hours after arrival at the altitude camp the test was repeated until the end of the 3-week stay. These data are based largely upon 2 subjects, since the third had to set up equipment in advance and was partially acclimatized at the time the tests could be started.

Table 3 indicates that the  $\text{CO}_2$  had already dropped nearly halfway to its final value at the end of a 12-hour exposure and plateaued off at the end of 4 days at this altitude where it remained constant. This progression is shown on the  $\text{O}_2\text{-CO}_2$  diagram in figure 3 and follows the predicted course except for the low R.Q. values. Not only was the pathway as predicted during the process of respiratory acclimatization, but also the final values after acclimatization fell exactly upon the predicted

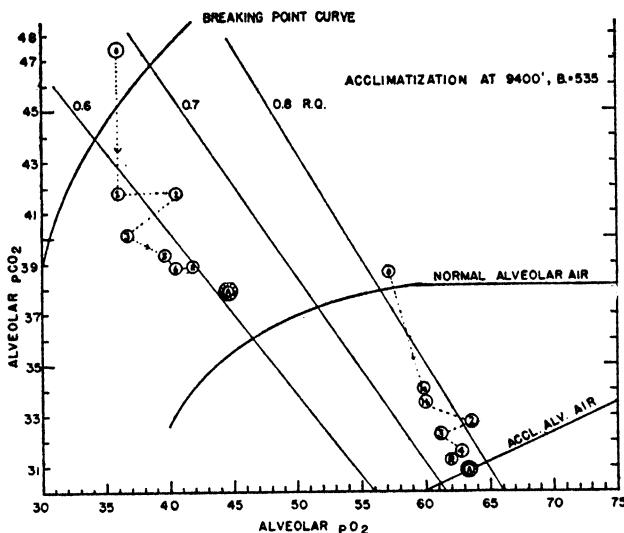


FIG. 3. NORMAL AND BREATH HOLDING BREAKING POINT alveolar air composition (dotted line) of 2 subjects during acclimatization at 9500 ft. The numbers in the circles indicate the days of residence at this altitude. The number 0 indicates 1 hour exposure and R the 5-18th day. 'A' indicates the average for all 3 subjects after respiratory acclimatization was complete. The 2 alveolar air curves (solid lines) are based on figure 2, while the breaking point curve is based on previous data (10) shown also in figure 7.

$\text{CO}_2$  level. Precisely the same change was recently observed by Hetherington, Luft and Ivy (14) when they reported an average difference of 7 mm  $\text{pCO}_2$  after acclimatization to 10,200 ft.

The fall of the alveolar R.Q. from an average of .84 to .76 (table 3) is of great interest. Although we have no satisfactory explanation, it should be pointed out that Hasselbalch and Lindhard (15) made similar observations during their 17-day stay at an altitude of 10,800 ft. Very recently the studies of Hetherington, Luft and Ivy (14) have confirmed these observations on 27 men who were taken from an elevation of 750 ft. to a 10,200-ft. level for a period of 2 weeks. The alveolar R.Q. was altered from an average of .80 to .75. Upon return to near sea level they observed a very slow return to the original value.

On the basis of the change in the alveolar  $pCO_2$  and R.Q. before and after acclimatization (last column, table 3), the predicted increase in alveolar ventilation should have been  $38/31 \times .76/.84$  or 11 per cent above the Rochester value provided that the resting oxygen consumption remained unaltered. Actually no significant change was observed. This could most easily be explained by a lower resting oxygen consumption at altitude. However, the studies and review of the literature by Lewis, Illif and Duval (16) indicate no change in basal metabolism in a comparison of 15 independent studies ranging from sea level to 7000 ft. In our subjects the number of breaths per minute was not altered, nor was there any appreciable change in the blood saturation as measured by the Millikan oximeter from that obtained during acute exposure at Rochester.

Averages for all 3 subjects at Rochester and after completion of respiratory acclimatization at Wyoming are given at the bottom of table 3. The rate of pulmonary

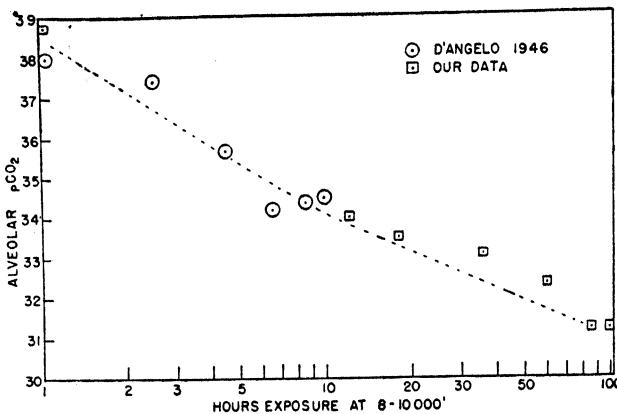


FIG. 4. RATE OF CHANGE of the alveolar  $pCO_2$  during acclimatization to an altitude of 8000-10,000 ft.

adaptation is best visualized in figure 4, in which the alveolar  $CO_2$  level is used as the index of acclimatization. This is completed by the end of 90 hours at this particular altitude. The rate must be governed by the degree of alkalinity incurred from the hyperventilation and the rate of base excretion from the kidneys. Our studies which start after 12 hours are well supplemented by those of D'Angelo (17) who exposed a large number of men in a high-altitude chamber to altitudes of 8 and 10 thousand feet for 10 hours. Although the alveolar gas was not determined directly in his studies, the  $CO_2$  can readily be calculated from the ventilation equation, since all the pertinent data were given, if one assumes a constant dead space. Thus, a fairly complete picture of the rate of acclimatization is obtained for the first time, since other expeditions were always forced to spend several days at various intermediate altitudes and thus became partially acclimatized before reaching their final destination.

*Respiratory Response of Man Acutely Exposed to Lower and Higher Oxygen Pressure after Acclimatization to Various Altitudes.* The only extensive data are those of

Hall and Wilson (4) who acclimatized 32 subjects to an altitude of 6200 ft. at Colorado Springs. These subjects were then exposed to various altitudes in a low-pressure chamber and their alveolar gases analyzed. Our data extend these observations to 9500 ft. where we breathed a mixture of 12 per cent oxygen in nitrogen after acclimatization (table 4).

The breathing of high oxygen mixtures after acclimatization causes no immediate change in the alveolar  $pCO_2$  and consequently no decrease in ventilation (table 4). This is in agreement with the recent work of Houston and Riley (8) who found that their subjects after being acclimatized to 22,000 ft. maintained their high ventilation and low  $CO_2$  value after being brought back suddenly to the relatively high-oxygen pressure of sea level. It would seem that re-acclimatization to sea level must occur slowly by retention of base in a similar process, but in reverse to that described above,

TABLE 4. RESPIRATORY RESPONSE TO 100% AND 12% OXYGEN AFTER ACCLIMATIZATION TO 9500 FT.  
ALTITUDE

INSPIRED GAS AT 9500 FT.	SUBJECT	NORMAL ALVEOLAR RESPONSE			BREATH HOLDING BREAKING POINT			
		$pCO_2$	$pO_2$	Oximeter	$pCO_2$	$pO_2$	Oximeter	Seconds
100% oxygen	A. O.	31.7		100	46.9	442 <sup>1</sup>	100	90
	R. S.			100	51.2	437 <sup>1</sup>	100	74
	H. R.	32.9		100	53.5	436 <sup>1</sup>	100	173
	Average	32.3		100	50.3	438	100	112
11.77% oxygen in nitrogen	A. O.	25.2	32.8	68	30.3	23.8	58	19
	R. S.	22.6	37.9	78	27.7	28.3	64	25
	H. R.			74	29.4	30.6	67	14
	Average	23.9	35.3	73	29.1	27.6	63	19

<sup>1</sup>  $pO_2$  values obtained by subtraction.

as demonstrated many years ago by Douglas *et al.* (18) upon their descent from Pike's Peak.

Figure 5 attempts to summarize the alveolar response of man exposed to various oxygen tensions after acclimatization. The solid line represents the alveolar values of the acclimatized individual as seen in figures 1 and 2. Point A is the sea level value and the dotted curve originating at this point is the acute response to low and high oxygen pressures. Point B is the alveolar value for man living at Colorado Springs at 6200 ft. and the dotted line again indicates the alveolar response when acutely exposed to lower oxygen pressures (4). Point C is the acclimatized point for approximately 10,000 ft., while the dotted line through this point indicates the response to lower as well as higher oxygen pressures. Similar curves could be drawn for other altitudes and the approximate alveolar response predicated as well as the ensuing acclimatization pathway. Thus as an example, if a man living at Colorado Springs were suddenly placed at an altitude of 18,000 ft. his alveolar pathway would start at B, jump to the intersection of the 18,000 ft. diagonal with the alveolar curve (dotted line) originating at B. From there it would travel down the 18,000 ft. altitude diag-

onal to its intersection with the acclimatized curve (solid line). On the other hand, if returned from 6200 ft. to sea level his alveolar pathway would travel from B on the dotted line to the right until it intersects the sea level diagonal and from there proceed slowly upward to point A.

*Increased Sensitivity of the Respiratory System to CO<sub>2</sub> after Acclimatization. A)* *CO<sub>2</sub> inhalation.* The first experiment measured the ventilation response to CO<sub>2</sub> added to the inspired air. The percentage composition of the various gases inhaled are listed in table 5 and give approximately the same CO<sub>2</sub> tension in the moist inspired

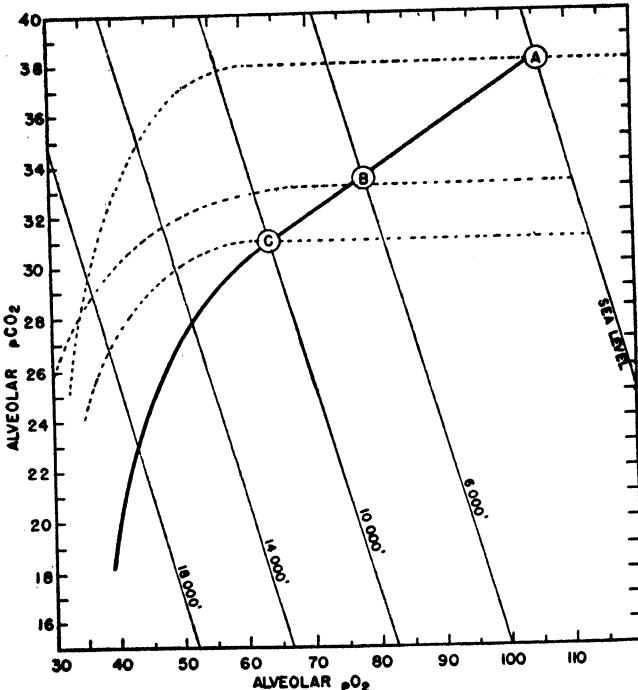


FIG. 5. ALVEOLAR AIR COMPOSITION of the acclimatized man (solid line) and the changes which occur (dotted line) when man acclimatized at sea level, (A); 6000 ft. altitude (B); and 10,000 altitude (C), is acutely exposed to lower or higher oxygen tension. See text.

air at both altitudes. The procedure was such that the subject inhaled consecutively air, low CO<sub>2</sub> and high CO<sub>2</sub> mixture each for 15 minutes from a demand regulator on each gas cylinder. Thus the change could be made instantaneously from one to the other without interruption. Minute to minute recordings were made of the alveolar gas composition, ventilation volume and rate, and ear oximeter. The last 5 minutes of each 15-minute exposure period were averaged. The 3 subjects were run on 2 occasions at both altitudes. The combined averages of all 6 runs are given in table 5.

Although the steady state was not attained with CO<sub>2</sub> breathing, as indicated by the low R.Q., the exposure time in all cases was the same and allows a comparison at

these 2 altitudes. Figure 6 indicates a greater ventilatory response for any given inspired CO<sub>2</sub> tension after acclimatization to 9500 ft. The ventilation increment is approximately 50 per cent greater over the range tested. Although breathing CO<sub>2</sub> increased the alveolar O<sub>2</sub> tension considerably it is expected that this in itself would not influence the response of the respiratory system during an acute exposure (see discussion above). If it did, the higher O<sub>2</sub> tension at altitude would tend to decrease the differences.

The sensitivity of the respiratory system might also be expressed in terms of change in alveolar pCO<sub>2</sub> which will produce a certain percentage increase in ventilation. If this is done for the high CO<sub>2</sub> mixtures at Rochester and Wyoming, we find that after acclimatization to altitude 0.75 pCO<sub>2</sub> induces the same ventilation increase as 1.00 pCO<sub>2</sub> at Rochester.

TABLE 5. EFFECTS OF CO<sub>2</sub> ADDITION TO THE INSPIRED AIR AT ROCHESTER, N. Y. AND AFTER ACCLIMATIZATION TO ALTITUDE. EACH FIGURE REPRESENTS AVERAGES OF TWO RUNS ON EACH OF THREE SUBJECTS (SEE TEXT).

INSPIRED GAS MIXTURE	INSPIRED pCO <sub>2</sub>	ALVEOLAR			OXIMETER	BREATHS PER MIN.	VENTIL. L./MIN. BTBS	VENTIL. RATIO
		pCO <sub>2</sub>	pO <sub>2</sub>	R.Q.				
<i>Rochester (B = 752 mm. Hg)</i>								
air	0	37.9	104	.86		11.7	0.59	1.00
3.2% CO <sub>2</sub>	22.5	40.2	135	.75		12.9	14.58	1.70
22.3% O <sub>2</sub>								
5.2% CO <sub>2</sub>	36.6	47.1	126	.75		12.7	23.92	2.78
19.7% O <sub>2</sub>								
<i>Wyoming (B = 537 mm. Hg)</i>								
air	0	30.4	65	.77	88	11.1	8.77	1.02
4.5% CO <sub>2</sub>	22.0	36.4	83	.71	92	12.0	16.98	1.98
20.6% O <sub>2</sub>								
7.0% CO <sub>2</sub>	34.2	41.5	91	.73	95	16.4	33.27	3.88
20.5% O <sub>2</sub>								

B) *Breath-holding tests.* The breath-holding tests were similar to those described by Otis, Rahn and Fenn (19). At the end of normal expiration the breath was held until the breaking point was reached and the alveolar air analyzed. The breath holding time and the ear oximeter reading were recorded. These data are summarized in table 3, while table 4 gives additional data for similar tests carried out breathing 12 per cent oxygen and 100 per cent oxygen after acclimatization was completed at 9500 ft. During the process of acclimatization the composition of the alveolar gas at the breaking point changes in much the same way as the alveolar gas before breath holding. This is shown by the 2 'pathways' plotted on the pO<sub>2</sub>-pCO<sub>2</sub> chart in figure 3.

On the basis of the various data in table 3 and 4 the breaking point curve and the normal alveolar curve after acclimatization have been drawn in figure 7 together with similar curves obtained previously on unacclimatized men (19). The vertical

distance between the alveolar air curve and the breaking point curve represents the magnitude of the  $\text{CO}_2$  stimulus necessary to achieve the breaking point for a given oxygen tension. Thus this distance may be regarded as measure of the sensitivity of the respiratory center to  $\text{CO}_2$ .

One can readily see that the vertical distance between these 2 curves is much greater at Rochester than after acclimatization to 9500 ft. ( $B = 535$ ). At the right hand side of the graph  $\text{CO}_2$  is the only factor concerned in achieving the breaking point, oxygen having no effect. Thus near sea level it takes a difference of about (65-38) 27 mm. Hg  $\text{CO}_2$  to reach the breaking point while after acclimatization to 9500 ft. at the identical oxygen pressure a difference of 18 mm. Hg (50-32) is sufficient. If we regard the breaking point as an expression of ventilation response (the same at both altitudes) then it is obtained by a change of  $\frac{1}{2}$  or .67 p $\text{CO}_2$  at 9500 ft. as compared with  $\frac{3}{4}$  or 1.00 p $\text{CO}_2$  near sea level. This figure agrees reasonably well with the relative stimulus of .75 obtained with  $\text{CO}_2$  inhalation (test described above).

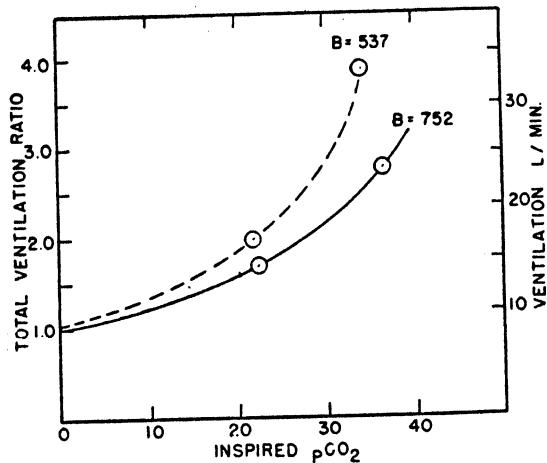


FIG. 6. EFFECT OF THE INSPIRED  $\text{CO}_2$  TENSION upon the total ventilation at sea level and after acclimatization to altitude. For details consult table 5.

From the  $\text{CO}_2$  dissociation curve one may estimate the  $\text{CO}_2$  content of the oxygenated plasma at a  $\text{pCO}_2$  of 38 mm. Hg and a  $p\text{H}$  of 7.4 to be 55 vol. per cent. If we assume that after acclimatization to 9500 ft. the  $p\text{H}$  returns to normal, then the  $\text{CO}_2$  content is reduced to 44 vol. per cent at a  $p\text{H}$  of 7.4 and a  $\text{pCO}_2$  of 31 (breathing pure  $\text{O}_2$ ). Under these conditions the computed  $p\text{H}$  at the breaking point for both altitudes is the same, namely 7.25. Thus under these conditions of breath holding with pure oxygen where oxygen lack does not enter as a stimulus it is very tempting to regard  $p\text{H}$  as the determining factor at the breaking point. Furthermore, the increased sensitivity of the respiratory system to  $\text{CO}_2$  after acclimatization might merely be the effect of this gas on the reduced buffering capacity of blood and tissues. This of course would also explain the continuation of hyperventilation when man acclimatized to altitude is suddenly exposed to higher than normal oxygen pressures.

C) *Breath-holding time.* From the above discussion it should follow that when a man acclimatized to various altitudes breathes pure oxygen and then holds his

breath, the breath-holding time is simply a function of his alkali reserve. Thus, from figure 2 one can estimate an alkali reserve reduction to about 80 per cent of normal at 10,000 ft. while the breath-holding time on pure oxygen in 2 of our subjects (R.S. and H.R.) was reduced to 70 per cent. Unfortunately, no further data are available.

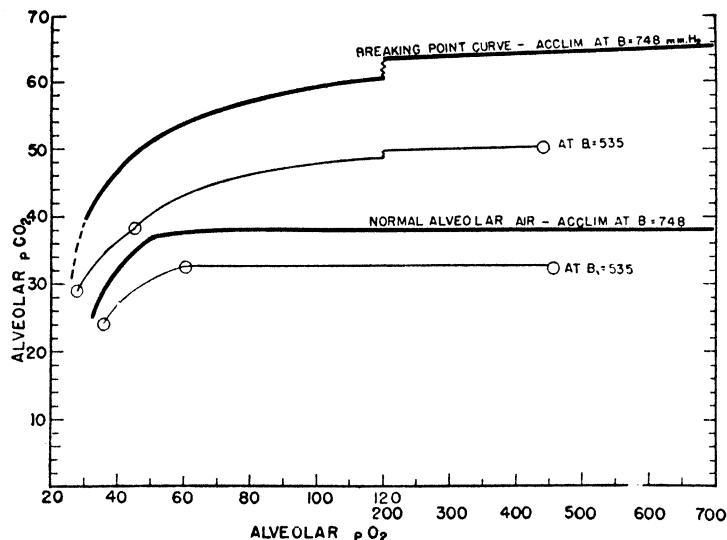


FIG. 7. NORMAL ALVEOLAR AIR COMPOSITION and that obtained after breath holding when man is exposed acutely to various oxygen tension. The heavy lines pertain to man acclimatized near sea level, the light lines after acclimatization to 9500 ft.

TABLE 6. BREATH-HOLDING TIME AT ALTITUDE (PERCENT OF SEA LEVEL VALUE)

EXPOSED ACUTELY <sup>1</sup>			ACCLIMATIZED													
Authority	No. of subj.		Altitude $\times 10^3$ Ft.					Authority	No. of Subj.	Altitude $\times 10^3$ Ft.						
			0	5	10	15	18			0	6	7	9.5	14	16	21
Engel et al (31)	40	100	82	68	59	54	54	Schneider (20)	1	100	55			33		
Rodbard (32)	80	100	94	76	72	56	56	Hingston (34, 35)	1	100		62		58	31	22
Otis et al (19)	8	100	81	66	59	54	54	Our data	3	100			50			
Brown (33)	212	100														
Average (not weighted)		100	86	70	63	56										

<sup>1</sup> The values at these particular altitudes were in part obtained by interpolation of the data.

When breathing air, however, the breath-holding time is in addition affected by the hypoxic stimulus. Even so the decreasing buffering capacity during the process of acclimatization should exert itself by reducing the breath-holding time as was long ago observed by Schneider (20). Table 6 attempts to summarize the recent investigation of breath-holding time during acute exposure to low oxygen. These data are in

very good agreement and are plotted in figure 8. When this curve is compared to the relatively few breath-holding data known for acclimatized man (20, 34, 35) a definite reduction in breath-holding time is indicated. This decrease in breath-holding time with acclimatization may to some extent be explained by the reduced buffering capacity but may in part be counteracted by a reduced sensitivity to low oxygen.

#### DISCUSSION

Experimental evidence and data from the literature are compared and indicate that the alveolar air composition and consequently the ventilation response of the respiratory system differ between acclimatized and unacclimatized man for any altitude above sea level. In general it may be stated that the acclimatized subject has a greater ventilation and consequently a lower  $pCO_2$  and a higher  $pO_2$  compared to an unacclimatized individual exposed acutely for one hour to the same altitude.

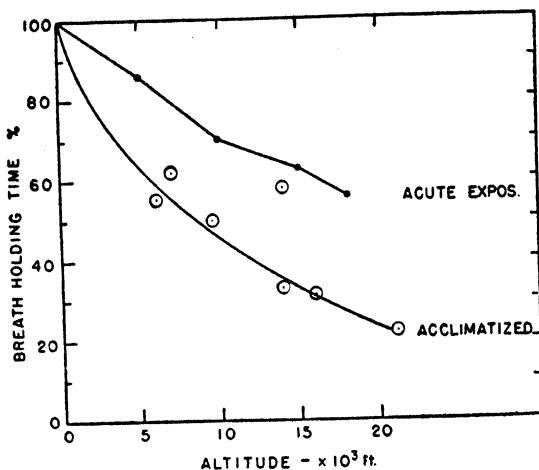


FIG. 8. BREATH HOLDING TIME as a function of altitude in man acutely exposed and acclimatized. Data taken from table 6.

If the rate of respiratory acclimatization is appraised by the alveolar  $CO_2$  level, then our data indicate that this process requires 4 days at an altitude of 10,000 ft. while the data of Douglas *et al.* (18) indicate at least twice as long at 14,000 ft. This ventilatory inhibition, which is thus gradually overcome during the adaptation process, is most simply explained by the respiratory alkalosis incurred from the hypoxic hyperventilation of the acute exposure. With the excretion of base the  $pH$  gradually returns to normal during the acclimatization process and the ventilatory inhibition is gradually diminished.

Direct and indirect (from alveolar air composition) measurements of the ventilation indicate no measurable change until man is acutely exposed to altitudes above 10,000 to 12,000 ft. At this altitude the alveolar and arterial  $pO_2$  tensions are between 50 and 60 mm. Hg. This is the approximate threshold response described by Schmidt and Comroe (21) for the chemoreceptors in dogs. On the other hand, v. Euler, Liljestrand and Zotterman (22), recording the activity of the sinus nerve, place

the oxygen threshhold much higher. This is supported by our curve of acclimatized man which indicates relative hyperventilation as soon as  $\text{pO}_2$  drops below sea level values (fig. 2). Thus we must look for an inhibition which temporarily (approximately 1 hour) prevents hyperventilation when man is acutely exposed to altitude less than 10,000 to 12,000 ft. or to equivalent oxygen pressures at sea level. The only apparent change is the desaturation of the arterial blood which brings about a small increase in the arterial  $\text{pH}$ . One might argue that not until secondary compensations take place which return the  $\text{pH}$  to normal level would the hypoxic stimulus be able to exert its effect. That such compensatory changes can occur in a relatively short time has been shown recently by Bjurstedt (23) who measured the  $\text{pH}$  continuously in dogs exposed to low oxygen.

Thus the conflicting views as to the hypoxic threshhold of the chemoreceptor may be reconciled by considering that this hypoxic drive upon the acute exposure up to approximately 10,000 to 12,000 ft. altitude (13-14%  $\text{O}_2$  at sea level) can be detected by an increase in action potentials from the chemoreceptor (22) but is masked by the desaturation alkalosis if measured by increase in ventilation volume (21).

However, when alveolar oxygen tensions lower than 50-60 mm. Hg are encountered the chemoreceptor drive becomes enough to overcome the inhibitory desaturation alkalosis and now produces an increased ventilation. The recent findings of Bjurstedt (23) that the chemoreceptor drive is actually potentiated by the resulting arterial alkalosis make it hard to explain the unresponsiveness in man acutely exposed to inspired oxygen tension above 13 to 14 per cent oxygen.

The maintenance of the relative hyperventilation at altitude has generally been attributed to the increase activity of the chemoreceptors. When after an acute exposure to altitude the subject is again returned to normal oxygen concentration the ventilation is immediately reduced to normal and even below normal depending upon the alkalinity incurred under the previous hyperventilation. In fact this subnormal ventilation after a stay at 22,000 ft. may last as long as half an hour (6).

However, if acclimatization to altitude has occurred, then a sudden removal to sea-level oxygen values does not reduce the ventilation immediately. This was long ago observed by Douglas *et al.* (18) and others, and most recently by Houston and Riley (8). Hyperventilation is maintained at sea level and gradually diminishes over a period of several days. This might suggest that after acclimatization to altitude the chemoreceptor activity is reduced and thus removal of the hypoxic stimulus has little effect upon the ventilation. Bjurstedt (23) has recently come to this conclusion in studies on dogs where he was able to differentiate between the centrogenic and chemoreflex control of ventilation during various stages of acclimatization. His findings indicate that the chemoreflex drive is all important during the acute exposure to hypoxia and is actually potentiated by concomitant alkalosis. After secondary compensations set in and return the arterial  $\text{pH}$  to normal, the chemoreflex drive diminishes and the centrogenic drive is supernormal and is largely responsible for the maintained hyperventilation after acclimatization to altitude.

Thus, the respiratory system response, according to this concept, is very similar to that at sea level with the exception that due to the lowered buffer capacity ( $\text{BHCO}_3$  content) it must, according to the Henderson-Hasselbalch equation, become more

sensitive to equal change in CO<sub>2</sub> as long as the  $p\text{H}$  is considered as one of the stimulatory factors acting upon the centrogenic drive.

Consequently, breathing pure oxygen after acclimatization to altitude or suddenly returning to sea level might reduce part of the already insignificant chemoreflex drive, but any reduction in ventilation will have a profound effect upon the acid-base balance and the immediate fall in  $p\text{H}$  and rise in pCO<sub>2</sub> will stimulate the respiratory center and thus prevent the drop in ventilation. Thus the same factor which operates to maintain a constant alveolar pCO<sub>2</sub> and arterial  $p\text{H}$  at sea level also operates to maintain the same arterial  $p\text{H}$  after acclimatization to altitude. The chemoreceptors play only a temporary part in the acclimatization process to high altitude in producing enough over-ventilation to lower the alkali reserve. Once this has been accomplished and the arterial  $p\text{H}$  has returned to normal, the centrogenic drive is able to maintain this hyperventilation by its greater sensitivity to CO<sub>2</sub>, this in turn being merely a reflection of the lowered buffering capacity. On such a basis one may find a ready explanation for the altered response of the respiratory system to inhalation of CO<sub>2</sub>, breath-holding time and breaking point concentrations described above. Gray (36), on the other hand, leans toward the idea that prolonged apnea as seen in altitude acclimatization increase the sensitivity to CO<sub>2</sub> per se, while the sensitivity of the respiratory system to  $p\text{H}$  remains unaltered.

If the chemoreceptor drive is relatively unimportant once acclimatization has been achieved then one should be able to simulate the respiratory responses of an altitude-acclimatized individual by any means which would induce a lowering of the alkali reserves at sea level. This could be achieved by prolonged hyperventilation or ammonium chloride ingestion. The effects of prolonged hyperventilation (24 hours) in a Drinker respirator have recently been reported by Brown *et al.* (37). They show in 3 subjects a considerable reduction in the serum bicarbonate level accompanied by an increased sensitivity to inspired CO<sub>2</sub> similar to the response reported in our observations at high altitude. Furthermore, after leaving the respirator involuntary hyperventilation was maintained for a considerable period as seen also in people returning from prolonged stays at high altitudes. Preliminary experiments in this laboratory have shown that daily ingestion of 15 gm. of NH<sub>4</sub>Cl for 3 days reduces not only the breath-holding time but also the alveolar pCO<sub>2</sub> at the breaking point of breath-holding in a manner similar to that observed after acclimatization to 10,000 ft. These experiments suggest, as has been pointed out by others, that a large part of the respiratory adaptations concerned with acclimatization to altitude is concerned with an adaptation to a lowered pCO<sub>2</sub> and a reduced buffer system of the blood which in turn raises the pO<sub>2</sub> tension and also prevents large fluctuations in O<sub>2</sub> tensions which are encountered during stresses such as work or voluntary apnea.

#### SUMMARY

With the aid of an O<sub>2</sub>-CO<sub>2</sub> diagram data are presented which allow one to predict the alveolar O<sub>2</sub> and CO<sub>2</sub> composition as well as the relative alveolar ventilation 1) when man is exposed acutely to any altitude, 2) during the process of respiratory acclimatization at any altitude and 3) when acclimatization is complete. In addition, the alveolar pathways can be predicted and described once man is acclima-

matized to any particular altitude and is then suddenly exposed to higher or lower oxygen pressures. These predictions were in part verified by an acclimatization study carried on at an altitude of 9500 ft. for a 3-week period.

Evidence is presented for a  $pO_2$  threshold of the chemoreceptor drive of ventilation at approximately 100 mm. Hg. This is observed in the ventilation curve of people acclimatized to altitude. However, in *acute exposures* where the alveolar  $pO_2$  is reduced to 50 to 60 mm. Hg this hyperventilation response is completely inhibited for at least one hour. This inhibition is explained as a result of the  $\text{pH}$  rise due to the decreased oxyhemoglobin saturation. If the alveolar  $pO_2$  in acute exposure falls below 50 to 60 mm. Hg, immediate hyperventilation occurs. Exposures of more than one hour at 9500 ft. result in a lowering of the  $pCO_2$  exponentially with time. Final levels are reached after 3 or 4 days.

After respiratory acclimatization to 9500 ft. the respiratory system becomes more sensitive to  $CO_2$ . This response was tested by breathing various  $CO_2$  mixtures and analyzing the alveolar air after breath holding. Data are presented which show the reduction of breath-holding time during acclimatization to various altitudes. The various findings emphasize that a large part of the respiratory acclimatization to high altitudes is an adaptation to a lowered  $CO_2$  tension.

The authors express their gratitude to Dr. W. O. Fenn, who in various ways has contributed much to this work.

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# INHIBITION OF BRAIN DEHYDROGENASES BY 'ANTICHOLINESTERASES'

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THE pharmacological actions of the anticholinesterase drugs, eserine sulphate (ES), diisopropylfluorophosphate (DFP) and tetraethylpyrophosphate (TEP), are usually attributed to their inhibition of cholinesterase (ChE). Some workers (1-3) have recognized that other actions might occur, and some (4-6) have found a lack of parallelism between pharmacologic effects and ChE inhibition. Other work in this laboratory (7, 8) has shown that these drugs inhibit the respiration of frog brain and nerve in concentrations which alter their electrical activity, as is also the case for methylfluoroacetate (MFA), which is not a ChE inhibitor. Yet the fluorophosphonates, tested on many purified enzyme systems *in vitro*, were found (9) inactive on all respiratory and other enzymes save only ChE and related esterases. It is, however, not exceptional for purified systems to be more resistant to damage than are less pure tissue extracts (e.g. 10), and even fluoroacetate was found (11) inert to most purified enzyme systems. We have accordingly tested these agents on brain and nerve brei, using the Thunberg methylene blue (MB) technique to reveal specific action on dehydrogenases. Marked inhibition of several of these has been found.

## METHODS AND RESULTS

Cattle sciatic nerves (obtained fresh at Swift and Co., courtesy of Mr. Keefer and Dr. Brewer) were cleaned, frozen, and finally powdered in dry ice; dog sciatics were homogenized (Potter-Elvehjem homogenizer) fresh in iced saline. Brains of adult white rats were excised immediately after decapitation, a portion used for dry-weight determinations, and the remainder homogenized (in a mortar by hand) in Ringer's solution. This was always completed in less than a minute. The suspension, made up to 20 mg. fresh tissue per ml. of Ringer's solution, was dialyzed in a cellophane bag against 10 volumes of Ringer, to remove substrates, by shaking at 130 strokes per minute for 3 hours at room temperature. To 1 ml. of dialyzed tissue suspension were added 0.3 ml. of substrate (analytic grade), 0.2 ml. of inhibitor, 0.1 ml. of 1:5000 MB, and physiological saline to a final volume of 2 ml. The pH was always approximately 7.0. Each Thunberg tube was at once evacuated, placed in a water bath at 37°C., and decoloration time noted. The percentage of inhibition or other ratio was calculated as:

$$100 - \frac{100 \times \frac{\text{minutes reduction time of substrate}}{\text{minutes reduction time of substrate plus inhibitor}}}{}$$

Received for publication March 1, 1949.

<sup>1</sup> Performed under contract between the Office of Naval Research and the University of Chicago.

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Early experiments were made on tissue kept frozen in dry ice, but this procedure was abandoned when it was found to injure the enzymes. This is shown in table 1. Except for succinate and  $\alpha$ -ketoglutarate in infant brain, all reductions are seriously retarded by freezing. Frozen cattle brain seemed somewhat more active than frozen dog brain; both were about  $\frac{1}{3}$  as active as fresh rat brain, and fresh nerve was only  $\frac{1}{10}$  as active as fresh brain. Table 1 also shows clearly the different rates at which various substrates are acted upon by a given brain homogenate, and that these various activities are altered in relation to each other by age and by freezing. Glutamate and fumarate seem to inhibit. Salts are important, and if water is used instead of isotonic saline, as in these early experiments, the activity of some dehydrogenases (citrate, glucose, lactate) is cut to 10 to 15 per cent (cf. 11a). Infant brain is defi-

TABLE 1. INFLUENCE OF AGE; INJURY BY FREEZING

*Non-dialyzed rat brain; in water*

	INFANT (3-DAY)			YOUNG (22-DAY)		ADULT			Frozen (5 days)	
	Fresh		Frozen (1 day)	Fresh		Fresh				
	13 23 2.9	12 23 2.7	12 23 2.7	16 23 3.7	18 23 4.1	23 20 4.7	23 9 2.1	23 9 2.1		
Dry wt., %.....										
Mg, wet wt/ml. susp.....										
Mg/dry wt/ml. susp.....										
<i>Addition (M/67)</i>										
Blank.....	53 <sup>1</sup>	130	180	20	45	36	180	180		
Oxaloacetate.....	27	22	50	11	11	5	47	180		
Succinate.....	17	21	20	7	8	6	10	60		
Glutamate.....	180	180	180	180	180	180	180	180		
Malate.....	32	42	68	16	11	23	52	180		
Citrate.....	50	60	78	23	21	19	120	180		
Lactate.....	26	34	77	8	14	14	140	180		
Glucose.....	29	40		18	9	17	55	180		
Pyruvate.....	24	39	78	17	18	13	58	180		
$\alpha$ -Ketoglutarate.....	45	64	60	25	25	21	120	180		
Fumarate.....	60	180		180	9	180	180	180		
Maleate.....	80	100	180	26	22	69	120	180		

<sup>1</sup> Reduction time in minutes. 180 = no reduction in 180 minutes.

nitely less active than adult on a wet weight basis, but is comparable in terms of dry weight (12).

Table 2 summarizes activities for particular substrates and rat brain under standard conditions. The independent behavior of each substrate strongly suggests that reduction rates are being determined by the activities of specific dehydrogenases rather than by some common factor, such as coenzyme (12a). That sufficient coenzyme was present so that it was not a limiting factor was further proven by adding coenzyme I (kindly supplied by Dr. F. Schlenk), 20  $\gamma$  per tube, to dialyzed suspensions. This did not hasten reduction time for substrates nor alter the inhibition produced by any inhibitor.

The critical experiments with inhibitors are summarized in table 3. Controls with hydrolysis products or associated ions of the inhibitors were negative. Sodium

fluoroacetate (NaFA) was practically inactive on brain or nerve, even when tested on homogenates in water with cells disrupted. It is striking that, for most substrates and at most inhibitor concentrations, the 'metabolic' inhibitor, MFA, is less effective against dehydrogenases than are the 'anticholinesterases', DFP and TEP. DFP is, on the whole but not uniformly (e.g. malate), somewhat more effective than TEP; ES is distinctly less so. The abilities of these drugs to inhibit tissue respiration *in vivo*

TABLE 2. RELATIVE ACTIVITIES OF SUBSTRATES WITH DIALYZED ADULT RAT BRAIN SUSPENSIONS

SUBSTRATE <sup>1</sup>	NO. OF TESTS	AV. REDUCTION TIME			RELATIVE ACTIVITY
		min.			
Oxaloacetate.....	6		3.4		100
Succinate.....	17		4.5		76
Malate.....	6		5.7		60
Lactate.....	14		6.1		56
Citrate.....	6		6.2		55
Glutamate.....	5		7.2		47
Glucose.....	6		7.2		47
$\alpha$ -Ketoglutarate.....	2		11.0		31
Maleate.....	2		15.5		28
Pyruvate.....	2		15.5		28
Fumarate.....	2		17.5		19
Blank.....	32		18.1		

<sup>1</sup> All substrates M/67. 20 mg. brain/ml.

TABLE 3

SUBSTRATE <sup>1</sup> (M/67)	PERCENTAGE INHIBITION OF MB REDUCTION																				
	M/45			M/200			M/450			M/800			M/1000			M/1500			M/10,000		
	ES	DFP	TEP	ES	DFP	TEP	MFA	ES	DFP	TEP	MFA	ES	DFP	TEP	MFA	ES	DFP	TEP	MFA		
Oxaloacetate.....		40	40	25	0				25	14	14	25	14	14	14	0	0	0	0		
Succinate.....	71	67	52	20	0	30	0	11	22	0	11	22	0	11	12	0	0	0	0		
Malate.....	73	76	73	40	0	50	59	37	31	52	28	26	33	31	0	0	0	0	21		
Lactate.....	86	100	100	18	0	70	47	15	56	45	0	41	15	0	17	0	0	0	0		
Citrate.....	50	60	39	14	0	10	39	14	5	26	14	0	7	14	0	0	0	0	14		
Glutamate.....	9	28	50	17	0	9	9	17	0	9	17	0	0	0	0	0	0	0	0		
Glucose.....	100	100	100	46	0	98	71	22	88	60	22	76	45	12	12	25	12	0	0		

<sup>1</sup> The substrates fumarate and maleate did not hasten MB reduction of undialyzed brain; pyruvate and  $\alpha$ -ketoglutarate were slightly active.

*vitro*, to inhibit ChE, or to produce symptoms in an animal are not in constant ratio to each other from case to case, even for the same phenomenon, so that extensive comparisons here would not be profitable.

There is little correlation between the activity of a dehydrogenase system and the ease with which it is inhibited by all these drugs, and, even at the highest drug concentrations used, the percentage inhibition for various substrates may range from 100 to under 30. Glucose is consistently the most fully inhibited, glutamate almost as regularly the least. Succinate is relatively more sensitive to DFP (and to ES)

than to TEP or MFA, especially at the lower range of inhibitor concentrations. Other particular instances of high sensitivity (malate to weak MFA) or low sensitivity (citrate to DFP) are manifest in the table. Dehydrogenases in the infant brain seem to be somewhat more susceptible than in the adult. In 2 experiments at  $10^{-4}M$  concentration, MFA gave about one-fifth inhibition with lactate and TEP about one-third. Dog nerve also showed significant inhibition of succinate and lactate oxidation by TEP, even at  $10^{-4}M$ . At  $0.005M$ , DFP and MFA inhibited succinate by 40 per cent, TEP by 60 per cent, and chloretone not at all.

In a few experiments, ribonucleic or desoxyribonucleic acid ( $200 \gamma/tube$ ) inhibited lactate dehydrogenase of brain by a third or more. (Compare liver, 13a.) A nucleic acid combined with one of the above drugs ( $0.001M$ ) roughly doubled the drug inhibition.

Of the systems tested, the dehydrogenase for succinate does not require coenzyme (or flavoprotein) for activity; all the others do. The latter, except for glucose, tended to be more easily inhibited.

TABLE 4. INHIBITION OF MIXED SUBSTRATES

SUBSTRATE	REDUCTION TIME <sup>1</sup> (MIN.)		% INHIBITION
	No addition	TEP $0.001M$	
Lactate.....	6.7	9.5	30
Succinate.....	5.5	5.5	0
Both.....	3.7	4.0	10
Both, calculated.....	3.1	3.6	15
Glucose.....	7.2	15.0	50
Succinate.....	4.2	4.2	0
Both.....	2.8	3.6	20
Both, calculated.....	2.6	3.2	20

<sup>1</sup> Values are adjusted for 4 to 6 comparable experiments using substrates at  $M/67$ ,  $M/144$ , or mixed concentrations.

tamate, tended to be more easily inhibited. That the drug action is not on coenzyme is shown, however, not only by the lack of inhibition of the glutamate system, but also by the earlier-mentioned inability of added coenzyme to alter the picture. Further evidence that the inhibitors act on the apoenzyme is supplied by their behavior with mixed substrates.

When either lactate or glucose, both of which are inhibited by  $0.001M$  TEP, is mixed with succinate, which is not inhibited, reduction time is shorter than that for either alone, but rather greater than that calculated for their complete summation  $\left( \frac{I}{t_A} + \frac{I}{t_B} = \frac{I}{t_{A+B}} \right)$  (table 4). This indicates that the two reducing systems are largely, but not completely, independent. If  $A$  is partially inhibited by a particular drug and  $B$  is not, reduction time for  $A + B$  should approach that for  $A$  by a calculable amount when no interdependence exists. Actually, as table 4 shows, for lactate or glucose as  $A$  and succinate as  $B$ , this is roughly the case. Results are alike whether the inhibited or the uninhibited substrate is added first. It thus appears that TEP can act on one dehydrogenase, leaving another unaffected.

## DISCUSSION

Apart from the poisoning of heavy metal proteins with cyanide or azide, two modes of action of inhibitors on respiratory enzymes have been described. Chloretone and barbiturates leave the dehydrogenases largely unimpaired, and probably affect a flavoprotein acting on cytochrome (13, 14). Morphine, codeine and thebaine, on the other hand, inhibit certain dehydrogenases of brain (15); this action has been suggested for diethylstilbestrol (16). The drugs here tested act also on the dehydrogenases. Whether several steps are involved in the substrate oxidation, as may well occur with glucose or oxaloacetate, or only one, as for lactate, is not important for the present findings. What is essential is that drugs regularly identified as anticholinesterases, and studied almost solely in terms of this property, are here shown to be powerful dehydrogenase inhibitors as well. Their ability to alter physiological behavior of nerve and brain follows their action on respiration more closely than their action on ChE (7, 8). It seems unjustifiable to attribute pharmacological effects specifically to changes in the acetylcholine system.

## SUMMARY

The dehydrogenase activity of brain and nerve was studied with the methylene blue technique. Relative activities with 11 substrates are presented. Age had little influence on brain dehydrogenases; freezing decreased activity to about one-fifth. Nerve was about one-tenth as active as brain. The 'anticholinesterase' drugs, DFP, TEP, and ES, are inhibitors of brain and nerve dehydrogenases, the two former being in general even more powerful than the 'metabolic inhibitor' MFA. Dehydrogenase inhibition is specific in pattern for each drug; even at  $10^{-4}$ M drug concentration the oxidation of particular substrates may be inhibited by 10 to 25 per cent.

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# DESCENDING RESPIRATORY PATHWAYS IN THE CERVICAL SPINAL CORD

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IT IS generally accepted that under normal conditions the activity of each half of the diaphragm is a consequence of excitation by the ipsilateral phrenic motoneurons, which in turn are activated by impulses originating in the medullary respiratory center and passing via descending fibers which are uncrossed (*viz.*, make direct connection with their ipsilateral phrenic nucleus).

The existence of some crossed connections in this system was first demonstrated physiologically more than fifty years ago by Langendorff (1) and Schiff (2). They showed that under certain experimental conditions the respiratory discharge descending on one side of the cervical spinal cord can excite the contralateral phrenic motoneurons. Porter (3) further demonstrated that this crossing occurred at the level of the phrenic nuclei. In 1912, Deason and Robb (4), working under the direction of A. J. Carlson, reported that the phenomenon of crossed excitation is a function of the intensity of discharge from the bulbar center. Still more recently, Rosenblueth and Ortiz (5) repeated Porter's work, with some modification of his methods, and concluded that the crossed phenomenon reveals properties of neurone paths which differ in quality from the conduction of nerve impulses and, in direct contrast to Deason and Robb, found that with maneuvers which markedly augment the intensity of the respiratory effort (e.g. asphyxia) they failed to demonstrate any crossed activity under the conditions of their experiments. Pitts (6) suggests that some hitherto unknown properties of the central nervous system underly the crossed phrenic phenomenon of Porter and that its elucidation would be a valuable contribution.

After reviewing the literature which bears on the points under discussion, it was found that there is disagreement as to the functional importance of the crossed connections. In particular there is disagreement as to the conditions necessary for the effective excitation of phrenic motoneurons by impulses which traverse crossed pathways. The data presented here have some bearing upon these points. We have studied the activity of descending respiratory pathways as reflected by the activity in each hemidiaphragm. The method of recording diaphragmatic activity is the principal point of difference between our procedure and those of previous investigators. Rather than observing the movements of the diaphragm or making mechanical records of its contractions, we have utilized the action currents of the two halves of the diaphragmatic muscle, amplified differentially and visualized by means of the cathode ray oscilloscope. Employing this method we have studied the effects on diaphragmatic activity both of enhancing and of diminishing the respiratory effort in cats and rabbits immediately following partial transection of the cervical spinal cord above the level of the phrenic motor nuclei.

#### METHODS

Sixteen acute experiments were performed, 8 with cats and 8 with rabbits. Some of the preparations had been decerebrated under ether anesthesia by the method of Schmidt (7). Others had been anesthetized with sodium pentobarbital ('Nembutal' Abbott), or diallylbarbituric acid with urethane and monoethylurea, its route of administration, and its dose are given in the abbreviated protocols presented below (see also figs. 3 and 4).

The vagal nerves remained intact, except in one rabbit in which they were transected near the end of the experiment (*exper.* 1). After inserting a glass tracheal cannula, each preparation was placed in the prone position with neck flexed, a partial laminectomy of the axis performed, and a partial transverse section of the cord made at the lower half of the second cervical segment. The preparation was then placed in the supine position and a midline abdominal incision made caudad from the xiphoid cartilage. The xiphoid cartilage was raised and clamped, and the abdominal wall and liver were carefully retracted. This maneuver sufficiently exposed the abdominal surface of the diaphragm to permit the symmetrical placement in it of two pairs of small electrodes, one pair lateral to the central tendon on each side. The electrodes were short loops prepared from fine insect pins. Each pair was connected, by means of the fine (no. 40) insulated wire, with the input terminals of a condenser-coupled differential amplifier of short time constant. The amplified diaphragmatic action potentials from each hemidiaphragm were visualized by means of cathode ray tubes and photographed on running film (see figs.).

Each experiment consisted of recording and comparing the action potentials of each hemidiaphragm *a*) in the control state and *b*) during various procedures which alter the respiratory effort: artificial hyperventilation and lung inflation to depress it; and tracheal occlusion, rebreathing, and lung deflation to augment it.

Toward the end of each experiment one or both of two tests that will be described below were made to determine quantitatively the degree of independence of the simultaneous recordings from the two sides of the diaphragm. The upper cervical cord was then removed and placed in 4 per cent formaldehyde. The extent of the lesion at C<sub>2</sub> was determined subsequently by analysis of serial sections stained by the Lillie variant of the Weigert method (8). The lesions for each experiment are shown in figures 3 and 4, in which the minimal areas of indubitable transection appear in black. A few distorted fibers in an edematous and/or hemorrhagic matrix remained in the stippled areas, and it is plausible to believe that the injury to them was sufficient to block the conduction of impulses. It is, of course, possible that functional block extended somewhat beyond the limits of the black and stippled areas.

#### RESULTS

The findings from a typical experiment on a cat (*exper.* 1) anesthetized with sodium pentobarbital (30 mg./kg.) are illustrated in figure 1. As in all the figures, action currents from the right hemidiaphragm are shown in the upper tracing, those from the left hemidiaphragm, in the lower tracing. The time-line gives 1-second intervals and the voltage scale is as indicated. The tracing on the upper left shows the extent of the transverse lesion at C<sub>2</sub>, as revealed by the histological analysis of serial sections.

As in all the figures, the orientation is cord dorsum to the right, the right side of the cord above and the left below to correspond with the action current records. It will be seen that in this particular experiment the lesion involved almost the entire anterior and lateral columns on the left, and a portion of the right anterior column as well.

The diaphragmatic action potentials of two inspiratory efforts are shown in the control record (*a*). In addition, the incidental recording of the QRS complex of the electrocardiogram is visible. The noteworthy feature of this record is the significant diaphragmatic activity on the side of the cord lesion. The activity on the side of the lesion, and on the opposite side as well, could readily be augmented; record *b* shows that significant enhancement above the control level was induced by rebreathing for 8 seconds; and record *d* illustrates the prompt augmentation produced by lung deflation.

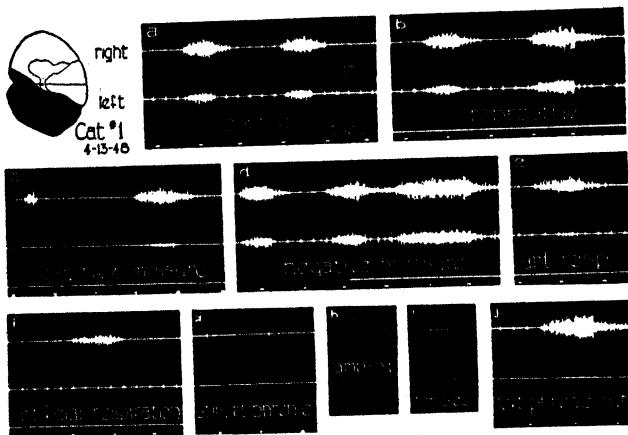


Fig. 1. ILLUSTRATIVE DATA from a typical experiment on a cat (pentobarbital anesthesia) *Upper left:* tracing of reconstruction of the partial transverse spinal cord section at  $C_2$ , oriented with cord dorsum to the right and right side at top. *Oscillograms:* action potentials from the 2 sides of the diaphragm, right above and left below to correspond with the drawing of the spinal cord. Time and voltage scales as indicated. Record *i* gives time-line for record *h*. See text for further details.

tion (application of negative pressure to the trachea). Likewise, the activity on both sides could readily be decreased. It is of particular interest that it proved easy differentially to abolish activity on the side of the lesion, that is, to abolish action currents on the side of the lesion while maintaining significant activity on the opposite side. One example may be seen in record *c*, which shows the first two inspiratory efforts that followed an apnea produced by lung inflation (positive pressure applied to trachea). The first inspiratory effort was almost entirely confined to the side opposite the spinal lesion; in the second, the activity on the side of the lesion was differentially reduced in comparison with the control state (record *a*). A second example is shown in records *e* and *f*. Hyperventilation artificially administered first produced differential reduction of activity on the side of injury (*e*), and then differential abolition of that activity (*f*). Continued hyperventilation eventually abolished activity

on the intact side. Cessation of hyperventilation was followed by similar changes in reversed sequence.

An essential control was determination of the independence of the simultaneous action current recordings from the two sides of the diaphragm. Accordingly, one or both of two tests were made in each experiment. Both tests were performed in the experiment now being described.

The first is illustrated by records *g* and *h*. Like records *a-f* and *j*, record *g* was taken on running film. Record *h* represents a single, relatively fast sweep, for which the time-line is shown in *i*. Both *g* and *h* were taken at one-tenth the standard amplification for the experiment as shown in *a*. The deflections show the action currents

TABLE I. SUMMARIZATION OF DATA

## Cats

Essential data from each of the experiments are summarized in tables 1 and 2 and in figures 3 and 4.

NO.	DATE	EXPER. PREPARATION AND CONDITION	EXTENT OF LESION GREATER OR LESSER THAN HEMI-SECTION (FIG. 3)	CROSSED ACTIVITY PRESENT IMMEDIATELY AFTER LESION	CROSSED-ACTIVITY CAPABLE OF BEING:		PERCENTAGE INTERACTION BETWEEN DIFFERENTIAL AMPLIFIERS
					ENHANCED OR BROUGHT IN	ABOLISHED	
1	4-13-48	Sodium pentobarbital; good condition	=	Yes	Yes	Yes	ca. 2.5
2	4-23-48 A	Sodium pentobarbital; good condition	<	Yes	Yes	Yes	< 1.7
3	4-23-48 B	Sodium pentobarbital; poor condition	<	Yes			< 2
4	4-24-48	Sodium pentobarbital; good condition	>	Yes	Yes	Yes	< 3
5	4-27-48	Sodium pentobarbital; good condition	>	Yes	Yes	Yes	1
6	5-3-48	Sodium pentobarbital; good condition	>	Yes	Yes	Yes	3
7	4-30-48	Decerebrated, good condition	>	Yes	Yes	Yes	< 2
8	5-11-48	Decerebrated; good condition	>	Yes, but small	Yes	Yes	< 0.3

produced by application of single shocks to the phrenic nerve in the neck on the intact (right) side. In both *g* and *h*, the large action current produced in the right hemidiaphragm was reflected by only a small deflection in the recording device connected with the left hemidiaphragm. The second test is section of the phrenic on the side of the cord lesion. As shown in record *j*, a large inspiratory effect on the intact side then produced little or no deflection in the record from the side corresponding to the cord lesion. In this experiment the interaction was conservatively estimated to be 2.5 per cent. It was considerably smaller in many of the other experiments.

It may be noted that the small or negligible amount of interaction had three implications: 1) physical spread of action current from muscle fibers in one hemidiaphragm to the recording electrodes in the opposite hemidiaphragm was small or

negligible; 2) physical interaction between the two channels of amplification was small or negligible; and 3) there was no evidence that fibers of the phrenic nerve cross peripherally (cf. Rosenblueth and Ortiz).

Results from an experiment on a rabbit (*exper. 6*) anesthetized with urethane are presented in figure 2. The diagram shows that the spinal lesion on the right side at C<sub>2</sub> was somewhat less than a hemisection. Attention may be directed first to the controls in which the degree of independence of the recordings from the two sides of the diaphragm was tested (records *f-j*). Record *f* shows that very large action potentials in the left hemidiaphragm, produced by stimulation of the left phrenic, produced scarcely visible deflections in the recording from the right. (The downward deflection produced by each volley in the left record was so large and rapid that it did not photograph sufficiently well to be reproduced.)

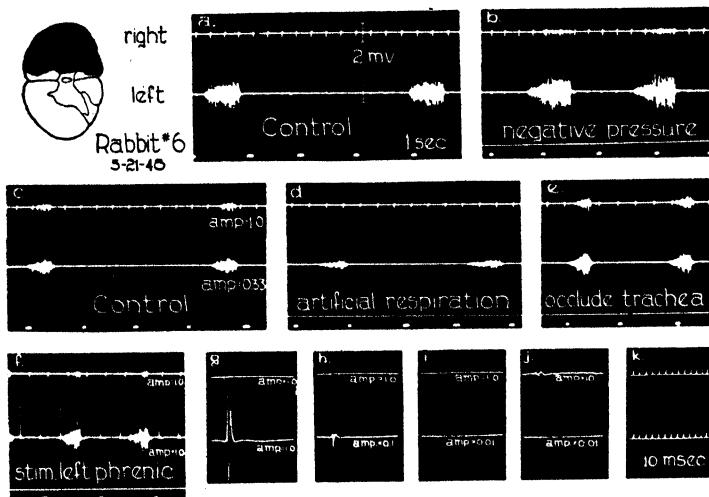


Fig. 2. ILLUSTRATIVE DATA from a typical experiment on a rabbit (urethane anesthesia). Arrangement as in fig. 1. Record *k* gives time-line for records *g-j*. See text for further details.

The degree of interaction may be determined more quantitatively from records *g-j*. At the standard amplification of the experiment (record *g*), stimulation of the phrenic on the intact (left) side evoked in that side of the diaphragm an action potential so large that it was only partially recorded. Nevertheless, only a minimal deflection followed the shock artefact in the record from the opposite (right) hemidiaphragm. In the next two records the amplification on the intact side was reduced, first 10-fold (*h*), then 100-fold (*i*), while that on the side of the cord lesion remained unchanged. It may be seen that the response on the intact side to stimulation of its phrenic, when reduced 100-fold, was still about three times as great as the deflection which appeared in the records from the opposite side as shown in the upper tracings of records *g*, *h*, and *i*. When the amplification on the intact side was reduced 100-fold and that on the opposite side simultaneously increased 10-fold, as shown in record *j*, the response on the intact side was approximately three times the greater.

Thus the controls agree in showing that the interaction in this experiment was only about 1 in 300, or 0.3 per cent.

As shown in record *a*, at the beginning of this experiment diaphragmatic activity in the control state was confined to the hemidiaphragm contralateral to the spinal lesion. However, lung deflation produced by application of negative intratracheal pressure, as shown in record *b*, promptly induced activity on the side of the lesion. As a sequel to very prolonged lung deflation with accompanying rebreathing into a small dead space, the preparation went into a circulatory and respiratory crisis with apnea. It quickly recovered during a short period of artificial respiration. Subsequently, and for the duration of the experiment (30 min.), diaphragmatic activity was present on the side of the lesion in the control state (record *c*). This crossed

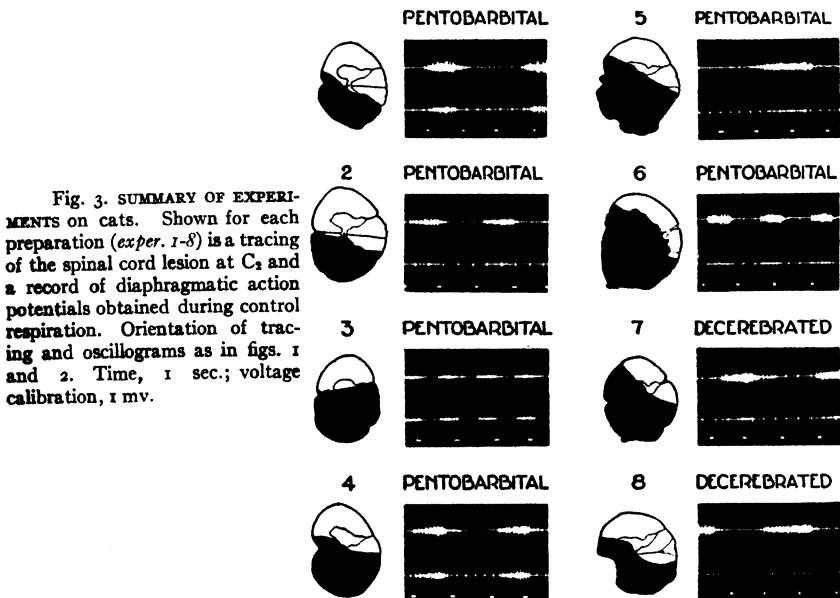


Fig. 3. SUMMARY OF EXPERIMENTS on cats. Shown for each preparation (*exper. 1-8*) is a tracing of the spinal cord lesion at C<sub>2</sub> and a record of diaphragmatic action potentials obtained during control respiration. Orientation of tracing and oscilloscopes as in figs. 1 and 2. Time, 1 sec.; voltage calibration, 1 mv.

activity could readily be differentially abolished during artificial hyperventilation (record *d*), and also during the first inspiratory efforts that broke through an apnea produced by lung inflation. It could be augmented significantly above the control level during the dyspnea produced by tracheal occlusion (record *e*), and also by forced deflation of the lungs.

#### DISCUSSION

*Cats.* In every experiment a significant amount of activity in the hemidiaphragm on the side of the spinal injury occurred during control respiration. The spinal lesions at C<sub>2</sub> varied from slightly less than a hemisection (*exper. 2*) to such an extensive injury as to leave intact only a part of the opposite lateral column (*exper. 6 and 7*).

In every experiment the crossed activity could be differentially abolished (i.e.

abolished with persistence of activity on the intact side) by artificial hyperventilation, or by forcible inflation of the lungs, or by both. These procedures, whatever else they may do, decrease the respiratory effort. In every experiment the crossed activity could be considerably augmented by rebreathing, forcible deflation of the lungs, and/or tracheal occlusion. These procedures, whatever else they may do, enhance the respiratory effort.

*Rabbits.* The rabbits differed quantitatively from the cats in that diaphragmatic activity on the side of the spinal lesion was initially present during control respiration in only 2 of the 8 preparations. Crossed respiration was present in 4 of 8 if one

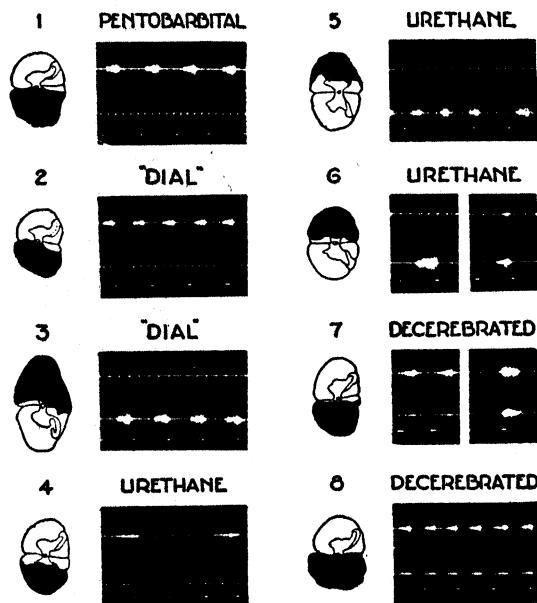


Fig. 4. SUMMARY OF EXPERIMENTS on rabbits. Arrangement same as fig. 3 except that voltage calibration indicates 2 mv.

includes *experiments 4 and 6*, in which crossing was initially absent but appeared during the course of the experiments.

In every case in which crossed respiration was absent during control periods, it could be induced in significant degree by the above-mentioned procedures which enhance the respiratory effort. In every case except one<sup>1</sup> in which crossed respiration was present in the control state, it could be differentially abolished by procedures which decrease the respiratory effort.

Bulbo-spinal fibers of respiratory function descending on each side of the cervical spinal cord make direct or indirect connections with neurons of both phrenic motor nuclei. From the standpoint of excitatory function, the connections with the crossed

<sup>1</sup> *Experiment 8* comprises a single exception to this statement. The respiration of the decerebrated preparation was irritative, presumably because of the presence of a blood clot found about the medulla at autopsy, and was very insensitive to reflex and chemical influences.

phrenic motoneurons are similar to but quantitatively somewhat less powerful than the connections with the ipsilateral motoneurons. Intensity of discharge from bulbar centers is a very important factor determining whether or not impulses in the crossing pathways effectively excite the crossed phrenic motoneurons. Effective excitation of phrenic motoneurons by impulses in the crossing pathways regularly occurs or can easily be induced, even when both the opposite phrenic nerve and the vagi are intact.

TABLE 2. SUMMARIZATION OF DATA  
Rabbits

NO.	DATE	EXPERIMENTAL PREPARATION AND CONDITION	EXTENT OF LESION-GREATER OR LESSER THAN HEMI-SECTION (FIG. 4)	CROSSED-ACTIVITY PRESENT IMMEDIATELY AFTER LESION	CROSSED-ACTIVITY CAPABLE OF BEING:		PERCENTAGE INTERACTION BETWEEN DIFFERENTIAL AMPLIFIERS
					Enhanced or brought in	Abolished	
1	4-15-48	Sodium pentobarbital; good condition	<	No	Yes		3
2	4-20-48	'Dial'; good condition	=	No	Yes		5±
3	5-28-48	'Dial'; good condition	=	No	Yes		0.3
4	5-19-48	Urethane; good condition	≤	No, but appeared spontaneously soon after	Yes	Yes	1
5	5-20-48	Urethane; good condition	<	No	Yes		0.3
6	5-21-48	Urethane; poor condition at first, then improved	=	No	Yes		0.3
7	5-14-48	Decerebrated; condition relatively poor due to clot about medulla	=	Yes	Yes	Yes	<11
8	5-18-48	Decerebrated; condition same as in no. 7	=	Yes	Yes	No	0.1

#### SUMMARY

In acute experiments on decerebrated or anesthetized cats and rabbits, a spinal lesion approaching or exceeding a hemisection was made at the second cervical level. The discharge of the phrenic motoneurons on each side of the cord was then measured by simultaneous action potential records from each hemidiaphragm. Controls established the absence of significant interaction between the 2 recordings.

In each of 8 cats significant crossed (i.e. ipsilateral to lesion) diaphragmatic activity occurred in the control state, even when the spinal lesion far exceeded a hemisection. This crossed activity could be completely and differentially abolished by procedures which decrease respiratory effort (e.g. hyperventilation), and augmented

by procedures which enhance respiratory effort (e.g. rebreathing, negative intratracheal pressure). Similar results obtained for those rabbits (4 of 8) which exhibited crossed respiration in the control state. Procedures which enhance respiratory effort elicited significant crossed activity in the remaining rabbits.

In conclusion, bulbo-spinal fibers of respiratory function descend on the intact side of the cord and make connections with phrenic motoneurons of the opposite side. These connections are quantitatively less powerful than the uncrossed ones. Intensity of discharge from the respiratory center is an important factor determining whether impulses in the crossing pathways alone will effectively excite the phrenic motoneurons.

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